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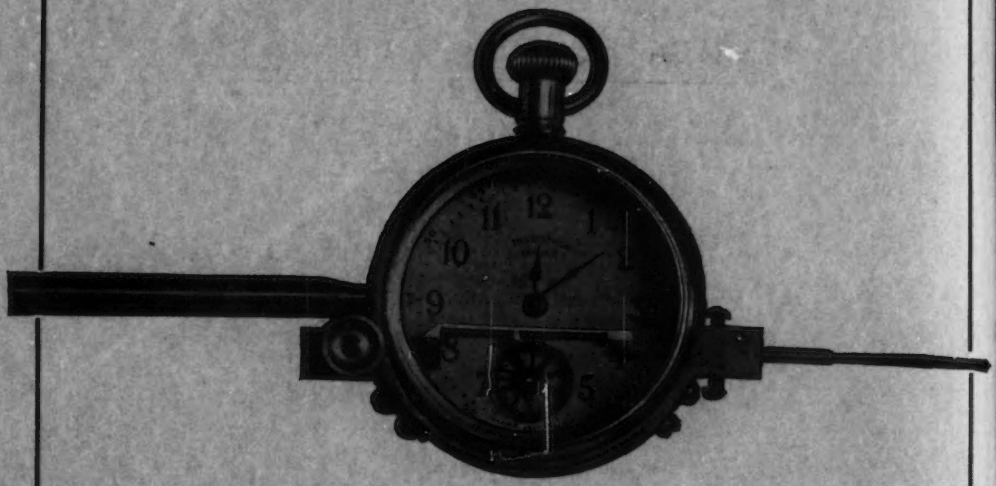

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THE EFFECT OF A RESTRICTED DIET

IV. On the Age of Greatest Productivity

JAMES ROLLIN SLONAKER and THOMAS A. CARD

From the Department of Physiology, Stanford University

Received for publication December 18, 1922

In our previous papers we gave the results of the effect of a restricted diet on growth (1), on the age of pubescence, the menopause, and on sterility (2), and on the number of litters and young born (3). In this paper we will discuss the effect of a restricted diet on the age of greatest productivity.

King (4) states that in stock albino rats and in a group of inbred rats the first litter was as a rule the smallest and the second litter the largest. She found the third and fourth litters intermediate in size between the first and second. Young females tended to be less prolific than older ones. The largest litters were produced when the females were about 150 days old. She found no great reduction in size of the litters until the females had passed the height of their reproductive activity, at about the age of 210 days. She also states that the size of any litter cast depends chiefly upon the age and physical condition of the female. As the experiment involved only the first four litters no data are given concerning later litters that would have been born had the pairs not been discarded. In a former paper King (5) has stated that the size of the average litter in rats at 3 months was 6.9, at 4 months 7.9, and at 15 months it was 4. That is, after the high point was reached there was a more or less gradual reduction in the size of the litter until they ceased to breed at about the age of 18 months.

That age has an influence on egg production has been shown by Dvorachek and Stout (6). They found the average egg production of the 1st, 2nd, 3rd and 4th year old hens was 72.17, 61.48, 50.53 and

24.57 respectively. Quite similar results were gotten by Carmichael and Rice (7) in breeding swine. They found that in young sows under 2 years of age the average litter was 7.5. In older sows it was 8.6 per litter. The average weight of the pigs of young sows was also less than that of older sows. They state that in successive litters from the same sow there was an increase in the size of the litters up to the fourth litter. Many other investigators have found similar results. These data show that the average ability to reproduce in the normal animal, when measured by the size of the litters cast, increases rapidly from puberty to a high point and then decreases slowly until the menopause, the last litter being the smallest litter thrown.

It is now our purpose to show how the normal curve of reproduction was modified in our rats which were fed on a diet restricted practically to plant sources.

In our first paper we gave in detail the manner of experimentation. In order to give a clear understanding it is deemed advisable to summarize briefly the method of procedure.

All groups of our rats were in the same room and subjected to the same external environment. They were all fed daily the same kind of plant food consisting largely of table scraps, green vegetables, nuts, cracked yellow corn, etc. Our controls had in addition animal protein from such sources as scraps of meat and eggs and some milk. The table scraps given to the restricted feeders were sorted over as carefully as possible, and bits of meat, eggs, etc., removed. However, some foods from animal sources could not be wholly eliminated, such as seasoning of butter, animal fats, milk, etc. With these exceptions, the diet of our restricted feeders was derived from plant sources. It corresponded fairly closely with the so-called vegetarian diet of human beings.

To facilitate handling the data we have divided our matings into five groups, based on the origin of the stock from which the individuals of the pair were taken. The control rats, or omnivorous feeders, have been designated group I. Those matings in which both male and female were selected from omnivorous stock and had been on an omnivorous diet until the time of mating were placed in group II. When one rat of a pair was taken from the control stock and its mate from the offspring of the restricted feeders, such matings constituted group III. All matings in which both sexes were selected from the young of restricted feeders and had been on a restricted diet since birth formed group IV. Group V was composed of those restricted feeders which toward the end of the experiment were given an omnivorous diet.

TABLE 1

Showing the average number of young and the average age of the parents for different litters

		LITTERS								
		1	2	3	4	5	6	7	8	9
Omnivorous (group I)	Number of pairs	70.0	49.0	49.0	31.0	17.0	10.0	8.0	5.0	2
	Number of young born	352.0	359.0	225.0	151.0	66.0	36.0	27.0	17.0	6
	Average young per pair	5.0	7.3	5.1	4.87	3.88	3.6	3.38	3.4	3
	Average age of parents	160.0	224.0	293.0	364.0	404.0	455.0	533.0	595.0	654
All matings on restricted diet (groups II, III, IV and V)	Number of pairs	62.0	39.0	16.0	2.0					
	Number of young born	299.0	162.0	54.0	11.0					
	Average young per pair	4.67	4.15	3.37	5.5					
	Average age of parents	264.0	353.0	439.0	549.0					
Omnivorous on restricted diet (group II)	Number of pairs	20.0	9.0	3.0						
	Number of young born	97.0	55.0	16.0						
	Average young per pair	4.85	6.1	5.3						
	Average age of parents	237.0	272.0	293.0						
1 omnivorous, 1 restricted on restricted diet (group III)	Number of pairs	8.0	8.0	4.0						
	Number of young born	32.0	40.0	15.0						
	Average young per pair	4.0	5.0	3.75						
	Average age of parents	264.0	357.0	495.0						
Restricted on restricted diet (group IV)	Number of pairs	10.0	6.0	5.0						
	Number of young born	43.0	22.0	15.0						
	Average young per pair	4.3	3.7	3.0						
	Average age of parents	225.0	325.0	441.0						
Restricted on omnivorous diet (group V)	Number of pairs	10.0	12.0	4.0	2.0					
	Number of young born	45.0	45.0	8.0	11.0					
	Average young per pair	4.5	3.75	2.0	5.5					
	Average age of parents	264.0	353.0	423.0	549.0					

By consulting table 1 it is seen that the average number of young per pair in the different groups varies with the age of the parents. In the omnivorous feeders (group I) it is observed that the average number of young per pair for the first litter was 5 and the average age of the parents was 160 days. The second litter averaged 7.3 per pair and the average age of parents was 224 days. From this point on there was a reduction in the average number of young per pair. If the number of young in the average litter is taken as a measure of the reproductive activity we find that in our normal rats there was an increase in ability to reproduce from sexual maturity to a maximum at the age of 7 or 8 months. After this there was a reduction, at first rapid, then gradual, over a period of 16 or 17 months when they ceased to reproduce. This is in general accordance with the findings of other investigators. The average number of young per litter, however, was less than that reported by other authors. This may be partly accounted for by the fact that some of the matings in our experiments were from animals well past the prime of life, some being as old as 390 days. Also, our control rats may not have had as rich a diet as those referred to at the beginning of this paper. As we shall show later, changes in the diet of the rat have a marked influence on reproduction. Figure 1 shows the results in a diagrammatic form. In this figure the ordinates represent the average number of young for each litter and the abscissae the average age in months of the parents at birth of each litter. It is seen that the interval between litters in the control rats was fairly constant, varying slightly between 2 and 2½ months, during the entire reproductive period. The average total reproductive activity extended over a period of about 17 months. This is longer than the average reported by others.

When we examine the results of groups II and III it is noticed that there was an increase followed by a marked decrease in ability to reproduce very similar to that found in the well-fed rats. The increase in reproductive activity, however, was only about half that of the normal. It is also seen that the maximum number of litters for each of these two groups was restricted to three and that the curves of these three correspond in general to that of the first three litters of the normal rats. The period of reproductive activity of group II was of about 2 months' duration, while that of group III was about 7 months. Why there should be a shortening of the time between litters in group II and a lengthening of it in group III as compared to the normal is at present uncertain. The immediate effect of a restricted diet on rats is to delay

sexual maturity. In group II it is delayed 3 months, and in group III over 4 months. When those animals which had been previously well fed (group II) did start to breed after the period of delay the interval between litters was very much shortened as compared to the normal. The later effect of a restricted diet seems to have been that of retardation or complete inhibition of reproductive activity. The figures of groups IV and V seem to confirm this latter assumption. When the total

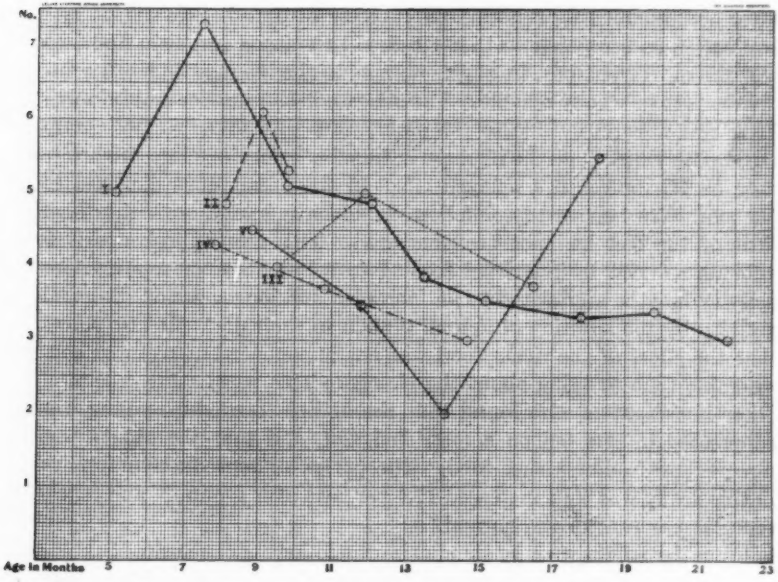

Fig. 1. Showing the average number of young and the average age of the parents at different litters. The ordinates represent the number of young and the abscissae the age of the parents. The numerals indicate the different groups. The heavy continuous line, *I*, represents our controls.

reproductive periods of groups II and III are compared to that of the normal, we find that in group II it represents but 13.7 per cent and group III only 45 per cent of the normal period. Comparing group IV with the normal we find a marked difference. The average maximum number of young was found in the first litter. This was followed by a gradual reduction in the average number born in the succeeding litters. That is, there was no increase in reproductive activity after the first litter

similar to that of the normal or of groups II and III. The curve shows a decline similar to the last part of the normal curve. This seems to indicate that the continued restricted feeding brought on sexual old age in relatively young rats. The duration of reproductive activity in this group was 7 months, which is but 43.6 per cent of the normal. This corresponds very closely with group III in this respect.

Group V corresponds fairly well to group IV until after the third litter. After this there was a rapid increase in the number born in the fourth litter accompanied by a noticeable increase of time between these last two litters as compared to that between litters one and two, and two and three. Litter four, however, represents the average of too few individuals to be wholly reliable. The increase in the number in this fourth litter was apparently due to the omnivorous diet given to this group during this time, while the increase in time between these last two litters may be attributed to a delayed effect of the omnivorous diet and to old age. We believe reproduction would have ceased with the third litter if the diet had not been enriched. This group illustrates how animals reduced by restricted feeding to a low state of reproductive activity may be stimulated to an increased and longer period of sexual activity by the addition of animal protein to their diet. It also shows that the effect of the richer food was not immediate but required considerable time to bring about the change. This verifies the results of other investigators who found that the ability to produce young was suppressed in rats fed on a restricted diet, and that the ability was restored by feeding richer food. The period of reproductive activity in this group was thus increased 11.4 per cent over group IV and represents 55 per cent of the normal.

From these data we may summarize the following conclusions:

1. In our control rats the young individuals threw smaller litters than older ones. In the restricted feeders variable results were obtained.

2. The second litter of our control rats was the largest and was cast when the parents were about 7 months old. In two of the restricted groups (II and III) the second litters were the largest and born at the average ages of the parents of approximately 9 and 12 months respectively. In groups IV and V on restricted feed the first litters were the largest and born when the parents were about 8 and 9 months of age respectively.

3. The greatest number of litters in our omnivorous feeders was nine. The average number of young for each litter in serial order was 5, 7.3, 5.1, 4.87, 3.88, 3.6, 3.38, 3.4 and 3. The lower average size of these

litters as compared to the reported size by other investigators we attribute partly to the average older age of the parents, but mainly to the difference in diet.

4. The average number of litters of each of the groups on the restricted diet was three.

5. The interval between litters in our controls was fairly constant, ranging between $1\frac{3}{4}$ to $2\frac{1}{2}$ months. In the restricted feeders it showed marked variations, ranging between about $\frac{3}{4}$ month and $4\frac{1}{2}$ months.

6. The period of reproductive activity in our omnivorous feeders extended over approximately 17 months. In the different groups of restricted feeders it ranged from less than 2 months to about 7 months.

7. Group V showed that rats which had practically ceased to breed at the third litter were rejuvenated by feeding food from animal sources so that they threw a fourth litter which was considerably larger than any they had cast on the restricted diet. The marked delay in the birth of the fourth litter emphasizes the statement that the effect of a change in diet is often not manifested for some time, sometimes representing a considerable portion of the life span of the animal.

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STUDIES ON THE MIXTURE OF AIR IN THE LUNGS WITH VARIOUS GASES. I

CHRISTEN LUNDSGAARD AND KNUD SCHIERBECK

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Complete mixture of the air in the lungs with other gases is a necessary condition for the validity of several methods of importance to physiology and pathology. Only few investigations have been undertaken in order to bring out the possibility of obtaining such mixture and none are decisive as to the laws governing the establishment of an equilibrium between lung air and gas from the outside.

Only very limited and rather contradictory conclusions can be drawn from the experiments of Gréhant (1), Bohr (2), Siebeck (3), Bruns (4), Sonne (5, 6), Krogh and Lindhard (7), Lundsgaard and Van Slyke (8). We have therefore subjected the problem to a systematic study, the results of which we shall report in this and in a following paper.

The gases generally used in methods requiring a mixture between lung air and one or more of these gases are: oxygen, hydrogen, nitrous oxide and carbon monoxide. Systematic experiments on mixture can be made only with oxygen and hydrogen. Nitrous oxide and carbon monoxide, on account of their solubility in blood, cannot be used in elucidating the general rules for obtaining complete mixture. We know, however, that diffusion of gases is inversely proportional to the square root of the specific gravity of the gases. The specific gravity of hydrogen is 1, of CO 14, of O_2 16, and of N_2O 22. If, through experiments, we could find the laws for obtaining mixture of lung air with hydrogen and oxygen, we should by intrapolation probably be able to obtain some information about the other gases.

A. MIXTURE OF LUNG AIR WITH OXYGEN. *Preliminary experiments.* In our first experiments concerning mixture of oxygen with lung air, our procedure was the same as that generally used in determining the lung volume. In a 5 liter, soft rubber bag (fig. 2) 2 liters of almost pure (97 to 100 per cent) oxygen were introduced. From this bag the subject rebreathed a certain number of times. His nose

was closed with a clamp. The difference in percentage of nitrogen in the bag and in the lungs decreases in the course of rebreathing. At the time of full mixture the percentage of nitrogen is (theoretically) equal in bag and lungs. It is therefore to be expected that two samples drawn from the bag after a different number of respirations, will show a higher percentage of nitrogen in the latter than in the former sample, until mixture occurs. After this period the two samples will contain the same percentage of nitrogen. This simple procedure was first used in a series of experiments. The results of seven experiments all performed on the same normal individual and in the same manner are given in table 1. After a certain number of respirations, a sample of 25 cc. was drawn from the bag without disturbing the breathing, which was carried on from two to five respirations more. The experiment was then stopped and the gas in the bag analyzed. We found in all instances a higher nitrogen percentage in the last sample than in the first, even after 30 to 35 respirations. This result, which we found in numerous experiments on different individuals seemed to indicate that it was impossible or at any rate very difficult to obtain full mixture between air in the lungs and the gas in the bag. The possibility that it was due to diffusion of gases (oxygen in this case) through the rubber bag was investigated and excluded. However, certain features in the results of the experiments made it unlikely that the difference in the nitrogen percentage of the two samples was due to incomplete mixture. The difference is, as will be seen in table 1, practically

TABLE 1

Determinations of percentage of nitrogen in bag after varying number of rebreathings. Normal subject. Maximum depth of rebreathings. Rebreathing started from total inspiratory position

EXPERIMENT NUMBER	SAMPLE I		SAMPLE II		DIFFERENCE BETWEEN SAMPLE I AND II
	Number of respirations	Nitrogen	Number of respirations	Nitrogen	
		per cent		*per cent*	
1	4	58.71	7	59.30	0.59
2	7	61.01	9	61.40	0.39
3	9	61.85	14	63.40	1.55
4	15	60.80	20	61.49	0.69
5	20	61.71	25	62.12	0.41
6	25	62.75	30	63.18	0.43
7	30	61.74	35	61.91	0.17

independent of the number of preceding respirations. There is no decrease in the difference between the first and the second sample corresponding to the increase in the preceding number of respirations. The differences are practically equal in the 4th to 7th, 20th to 25th, and 30th to 35th respirations. This fact is not in accord with the law which the production of mixtures necessarily must follow. In figure 1 the curved line, *a*, represents in a schematic way the expected behavior of an experiment. The difference between the nitrogen percentage in different respirations becomes smaller and smaller until it disappears when mixture is obtained, that is, at the time when the curve becomes horizontal. The differences found in the experiments reported in

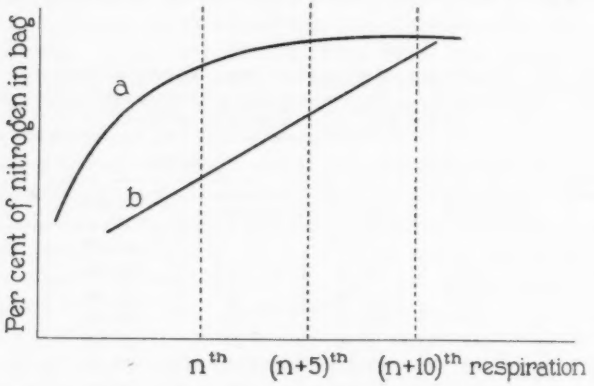

Fig. 1. Curve *a* indicates the percentage values of nitrogen one should expect to find in the bag in the course of a rebreathing experiment. Curve *b* indicates the values observed after the fourth respiration. (See table 1.)

table 1 can be due to incomplete mixture only if the law governing the mixture can be represented by a (practically) straight line, *b*, which is incomprehensible.

Conditions in the bag during rebreathing. In order to be able to undertake a closer study of the values of the nitrogen percentages in the bag during the experiment the technique was changed in such a way that samples could be repeatedly drawn from the bag after each expiration. Figure 2 shows the procedure. A number of lead tubes (8) with capillary boring were cemented together in a glass tube. The glass tube was then introduced into a side arm in the bag and kept in such a manner that no dead space was present.

Through these lead tubes several samples of gas from the bag were drawn during the course of the experiment. These were collected in evacuated samplers after an expiration into the bag. In the tables the time at which the samples were drawn is indicated by the number of the preceding respiration. If the experiment starts from the maximal inspiration (total capacity) of the individual, the number indicating the time at which the sample is drawn will be a fraction; if it starts from maximal expiration (residual air) the number will be an integer. In table 2 are reported a few of a number of experiments giving similar results. The first two experiments (nos. 37a and 37b) were performed on a normal individual. Five experiments (nos. 41a, 41b, 43a, 43b and 44) were done on patients suffering from emphysema pulmonum and with a history of bronchial asthma. At the time of the experiments no rales or dullness were observed in the lungs. The remarks in the tables probably give sufficient explanation of the conditions of the experiments.

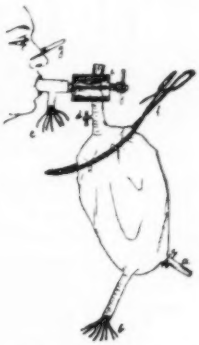

Fig. 2. Five-liter rubber bag. Lead tubes with capillary borings inserted at *c* and *b*. Through these tubes samples of air can be drawn into evacuated samplers without interrupting rebreathing. By means of the soft clamp the last part of the final expiration can be segregated from the rest of the expired air. *a* is used for filling the bag with oxygen or hydrogen mixture.

It is seen that the nitrogen percentage in the bag increases very rapidly from the zero point during the first two or three respirations. The increase is largest during the first one and a half respirations, smaller during the second, and still smaller during the third. At the time of (usually) the 4th and 5th respirations the increase in the nitrogen percentage of the bag is very small, so small that the values are almost constant. After this time the increase in the nitrogen percentage usually becomes somewhat larger. Apart from minor insignificant variations, this holds true in all our experiments.

In order to make it more clear, curves are constructed on the basis of the values from the nitrogen percentage in table 2. The curves (figs. 3 to 5) consist of three parts: first, a very steep ascent representing the conditions during the first three or four respirations; second, an almost horizontal part usually corresponding to respirations 4 and 5; and third, a last part where the upward movement is more marked

TABLE 2

Nitrogen and carbon dioxide in samples drawn from bag during experiment without disturbing the rebreathing. (See fig. 2)

EXPERIMENT NUMBER IN PROTOCOL	NUMBER OF RESPIRATION WHEN SAMPLE WAS DRAWN	NITROGEN IN BAG	CARBON DIOXIDE IN BAG	REMARKS
		per cent	*per cent*	
37a	4½	59.71	5.20	Normal subject. Total lung capacity (5.9 liters) in lungs when experiment started. Vital capacity 4.5 liters. Residual air 1.4 liters. Depth of rebreathings 3.5 to 4 liters. Respiration rate about 10. Two liters of oxygen in bag at the beginning of the experiment
	6½	60.06	5.76	
	8½	60.37	5.99	
	10½	61.38	6.55	
37b	4	29.66	3.645	Same subject as in experiment 37a. Experiment started from maximum expiration. Depth of rebreathings 2.5 liters. Three liters of oxygen in bag at the beginning of experiment. Respiration rate about 20
	7	32.12	4.54	
	9	32.23	5.61	
	10	32.60		
41a	4½	57.59		Patient with emphysema of lungs and bronchial asthma. Total lung volume 6.6 liters. Vital capacity 4.2 liters. Residual air 2.4 liters. Rebreathing started from maximum inspiration. Depth of rebreathing about 3.5 liters. Two liters of oxygen in bag at the beginning of the experiment. Respiration rate about 11
	5½	60.94		
	6½	61.84		
	7½	63.63	7.78	
41b	2½	56.61	3.94	Same subject and conditions as in experiment number 41a
	3½	58.64	4.95	
	4½	60.06	5.43	
	5½	60.97	6.16	
	7½	62.27	6.53	
	8½	62.68		
43a	½	(55.31)		Patient with emphysema of lungs and bronchial asthma. Total lung volume 5.9 liters. Vital capacity 3.6 liters. Residual air 2.3 liters. Rebreathing started from maximum inspiration. Depth of rebreathing about 3 liters. Two liters of oxygen in bag at the beginning of the experiment. Respiration rate not observed
	1½	56.13		
	5½	59.54		
	6½	59.53		
	7½	59.74	5.53	
	8½	60.00		

TABLE 2—*concluded*

EXPERIMENT NUMBER IN PROTOCOL	NUMBER OF RESPIRATION WHEN SAMPLE WAS DRAWN	NITROGEN IN BAG	CARBON DIOXIDE IN BAG	REMARKS
		per cent	*per cent*	
43b	1	26.91		Same subject as in experiment 43a. Rebreathing started from maximum expiration. Three liters of oxygen in bag at the beginning of experiment. Depth of respiration about 2.5 liters. Respiration rate not observed
	5	31.26	5.77	
	6	31.37	6.17	
	7	31.63	6.25	
	8	32.20		
44	½	56.53	3.25	Patient with slight emphysema of lungs and bronchial asthma. Total lung volume 6.5 liters. Vital capacity 4.8 liters. Residual air 1.7 liters. Rebreathing started from maximum inspiration. Depth of rebreathing about 4 liters. Two liters of oxygen in bag at the beginning of the experiment. Respiration rate not observed
	3½	60.34		
	4½	61.05	5.03	
	5½	61.08		

than in the middle portion. A curve of such a form cannot be caused merely by the mixing process of the lung air rich in nitrogen with the nitrogen-free gas in the bag. As previously mentioned, a curve representing the nitrogen in the bag during the experiment would run a course like curve *a* in figure 1. The first 3 to 5 respirations represent such a curve, the later respirations, however, do not. During subsequent respirations the increase in nitrogen percentage in the bag is almost constant from respiration to respiration. It is similar to the increase in our first experiments (table 1, and fig. 1, curve *b*). This indicates that during the rebreathing something else besides the mixing takes place in the closed system of the bag and lungs. This "something else" which is veiled during the first respirations causes, as all the experiments in table 2 show, an almost regular increase in nitrogen percentage in the bag.

Two circumstances may be the cause of this increase in the nitrogen percentage in the bag. One is, transition of nitrogen from blood to alveolar air on account of the decreasing nitrogen percentage within the lungs during the experiment. This factor, however, is probably too small to be the single cause of the continuous increase of nitrogen

percentage in the bag. The other factor is a decrease in the volume of the sum of oxygen and carbon dioxide in the bag plus the lungs. This decrease is caused by the fact that the oxygen intake usually exceeds the carbon dioxide output. If the decrease in the volume of gas in the

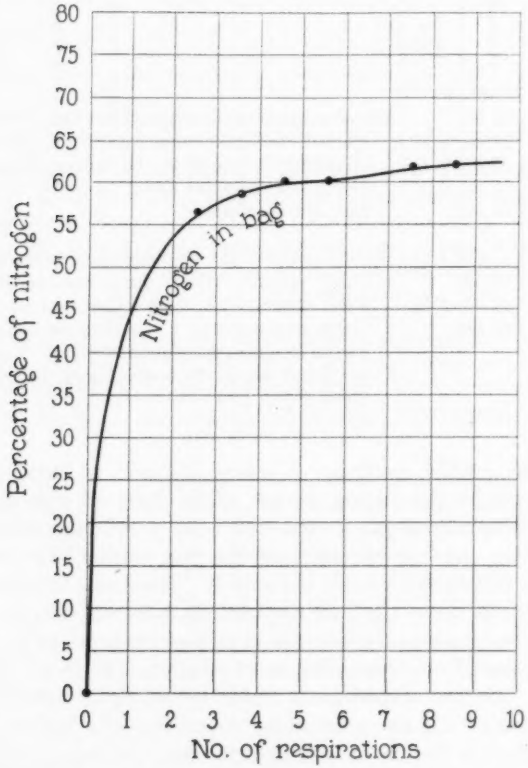

Fig. 3. "Mixing curve" showing the percentage values of the nitrogen in the bag during experiment 41b.

bag and lungs was due merely to the normal difference between oxygen intake and carbon dioxide output, the percentage of nitrogen would not be materially increased. However, two factors coöperate in increasing this difference. One is an augmented diffusion of oxygen into the blood because the oxygen pressure in the alveoli is higher than usual;

the other is a diminished diffusion of carbon dioxide from the blood to the lungs on account of the increased pressure of that gas in the alveoli. (It is a well-known fact that equilibrium between carbon dioxide pressure in the lungs and in the venous blood can be established in a similar way.) These factors all act in the same direction, to increase the

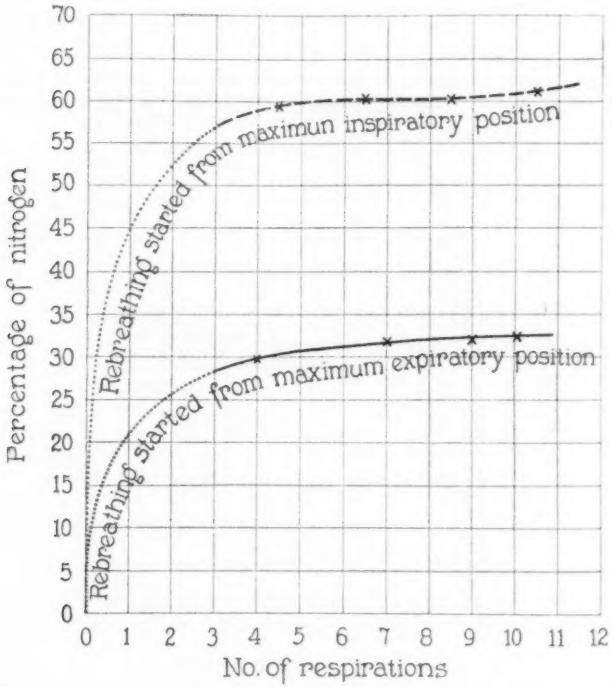

Fig. 4. Curves representing nitrogen values in bag when subject started rebreathing from maximum inspiratory position (exper. 37a) and maximum expiratory position (exper. 37b).

nitrogen percentage, first in the lungs, and subsequently in the bag. The changes in nitrogen percentage in the experiments in table 2 are, according to this, undoubtedly to be explained as follows: a full mixture between the air in the lungs and in the bag takes place during the first 2 to 4 respirations, corresponding to the first part of the curve in figure 3. When the mixture is complete, the result of the above men-

tioned two factors becomes visible and shows itself as a continuous, rather slight increase in the nitrogen percentage. This period corresponds to the two last parts of the curve. The increase may be large enough to give the impression that full mixture has not yet taken place; and more than this, it may be taken as proof that full mixture cannot

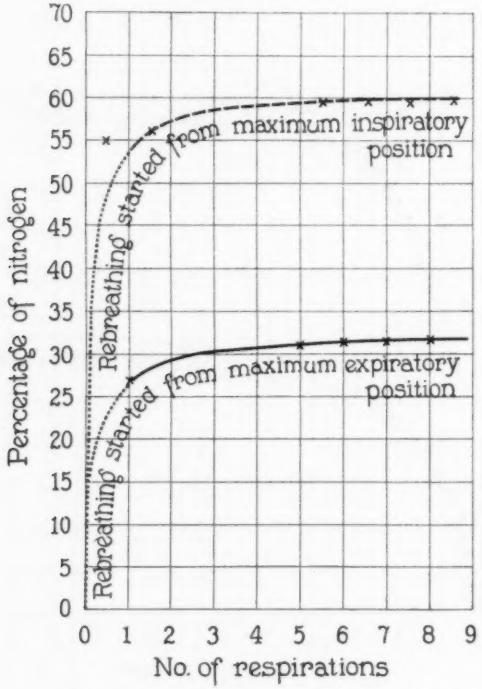

Fig. 5. Curves representing nitrogen values in bag when subject started rebreathing from maximum inspiratory position (exper. 43a) and maximum expiratory position (exper. 43b).

be obtained at all. In most of our experiments the subject has started the rebreathing from maximum inspiratory position of the chest. In a few, however, maximum expiratory position was the starting point. In this last instance the total amount of gas in the bag-lung system is of course much smaller, and the final nitrogen percentage lower. For these reasons we should expect a larger and earlier effect of the above

mentioned factors. That this is actually the case is shown in figures 4 and 5. The second, almost flat, part of the upper curves is not present in the lower ones, because the continuous increase in nitrogen percentage is already marked at the point where the effect of the mixing proper ceases.

Conditions in the lungs during rebreathing from the bag. In view of the preceding results obtained by analyzing successive samples from the bag, it is of interest to follow the conditions in the lungs.

In order to obtain samples of gas from the last part of an expiration without interrupting the experiment, the previously described glass

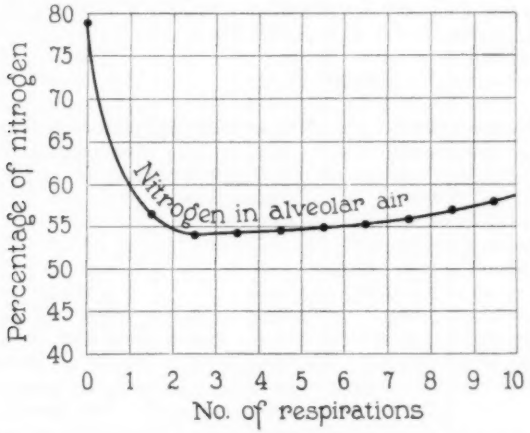

Fig. 6. Curve representing nitrogen values in the last part of expiration (alveolar air), experiment 50, table 3, when the upper curve bends upwards, mixture has taken place.

tube containing 8 capillary lead tubes is introduced in the side arm of a T-shaped mouth-piece of rubber (fig. 2). Samples from the last part of each expiration can now be collected into previously evacuated samplers without any disturbance of the experiment.

In table 3 the results of a number of experiments similar to those reported in table 2 are given. All the experiments show the same main features in the change in nitrogen percentage. During the first 2 to 4 respirations, the nitrogen percentage decreases rapidly from its original value of about 79 per cent. Then a slight steady increase of the nitrogen percentage is seen. In figure 6 a curve is drawn showing the changes

TABLE 3

Determinations of nitrogen and carbon dioxide in samples drawn from the very last part of expiration without disturbing the rebreathing. (See fig. 2)

EXPERIMENT NUMBER IN PROTOCOL	NUMBER OF RESPIRATION WHEN SAMPLE WAS DRAWN	NITROGEN IN ALVEOLAR AIR.	CARBON DIOXIDE IN ALVEOLAR AIR	NITROGEN IN BAG AT END OF EXPERIMENT	REMARKS
		per cent	*per cent*	*per cent*	
39	2½	65.45			Normal subject and condition as in experiment 37a in table 2
	3½	64.68			
	4½	64.85			
	6½			63.56	
41	2½	65.70	(5.01)		Patient with emphysema of lungs and bronchial asthma. Same patient and conditions as in experiment 41a in table 2. Respiration rate 9
	3½	63.25	(4.88)		
	4½	62.29	6.09		
	6½	63.22	6.97		
	7½	63.77	7.37		
	8½	64.42	7.45		
	10½			64.40	
42	3½	61.91	6.00		Normal subject. Total lung capacity 5.8 liters. Vital capacity 4.67 liters. Residual air 1.13 liter. Depth of rebreathing 3.5 to 4 liters. Experiment started from maximum inspiration. Two liters of oxygen in bag at the beginning of the experiment. Respiration rate not observed
	4½	61.11	6.20		
	5½	61.33	6.20		
	6½			61.04	
44	½	78.98			Same patient (emphysema of lungs and bronchial asthma) and conditions as in experiment 44, table 2. Respiration rate 13
	1½	63.13			
	3½	61.94			
	4½	62.16			
	5½			60.60	
49	2½	60.90			Patient with emphysema of lungs and bronchial asthma. Total lung capacity 5.5 liters. Vital capacity 3.3 liters. Residual air 2.2 liters. Rebreathing started from maximum inspiration. Two liters of oxygen in bag at beginning of experiment. Depth of rebreathings 2.5 to 3 liters. Respiration rate 11
	3½	60.41			
	4½	60.81			
	5½	61.40			
	6½	61.91			
	7½	62.39			
	8½	63.10			
	9½	63.41		62.60	

TABLE 3—*concluded*

EXPERIMENT NUMBER IN PROTOCOL	NUMBER OF RESPIRATION WHEN SAMPLE WAS DRAWN	NITROGEN IN ALVEOLAR AIR	CARBON DIOXIDE IN ALVEOLAR AIR	NITROGEN IN BAG AT END OF EXPERIMENT	REMARKS
		per cent	*per cent*	*per cent*	
50	1½	56.50			Patient was convalescent after rheumatic fever. Lungs normal. A systolic murmur was heard at apex of heart. Second pulmonic sound slightly accentuated. Size of heart was normal. Total lung volume 3.9 liters. Vital capacity 3.1 liters. Residual air 0.8 liter. Rebreathing started from maximum inspiration. Two liters of oxygen in bag at the beginning of the experiment. Depth of rebreathings about 2.5 liters. Respiration rate not observed
	2½	54.29			
	3½	54.40			
	4½	54.69			
	5½	55.00			
	6½	55.29			
	7½	56.10			
	8½	57.20			
	9½	58.49		56.89	
51	1½	54.59			Patient with mitral insufficiency and mitral stenosis. No rales heard over the lungs on the day of the experiment. Stasis of lungs previously observed on several occasions. Total lung volume 3.4 liters. Vital capacity 2.0 liters. Residual air 1.4 liters. Experiment started at maximum inspiration. Two liters of oxygen in the bag at the beginning of the experiment. Depth of rebreathings 1.5 liters. Respiration rate not observed
	2½	53.50			
	3½	52.11			
	4½	51.76			
	5½	51.92			
	7½			51.51	
54	4½	56.75			Patient with mitral stenosis. No stasis in the lungs. Total lung capacity 4.7 liters. Vital capacity 3.1 liters. Residual air 1.6 liters. Experiment started at maximum inspiration. Two liters of oxygen in bag at the beginning of the experiment. Depth of rebreathing 2.5 liters. Respiration rate not observed
	5½	57.21		56.40	

in nitrogen percentage in experiment 50. The results of all the experiments being practically uniform, this curve may be taken as typifying the changes in the nitrogen percentage in the lungs during mixing experiments. The curve consists of two parts and has an upward

concave form. The first part, which corresponds to the first 2 to 3 respirations, shows a very steep downward slope, the values for nitrogen percentage sinking from 79 per cent to about 54 per cent during the first 2½ respirations. At this point the second part of the curve begins. It shows a slight continuous ascent until the 9½ respiration, at which point the nitrogen has a value of about 58 per cent.

The first part of the curve corresponds closely to what we might expect would take place on account of the dilution of the lung air with the nitrogen-free gas in the bag. The last part, however, which re-

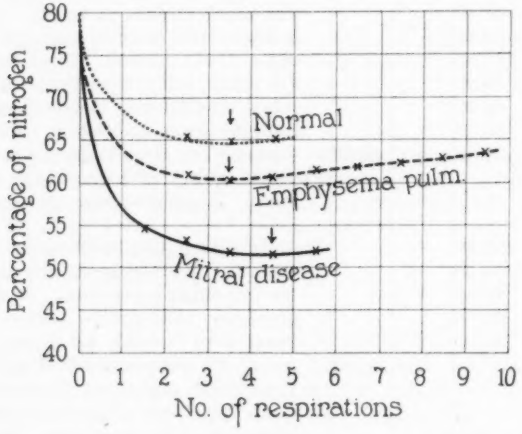

Fig. 7. Three curves representing nitrogen values in alveolar air during rebreathing in a normal subject (exper. 39, table 3), a patient with emphysema of lungs (exper. 49, table 3) and a patient with mitral disease (exper. 51, table 3). In all three cases the rebreathing started from maximum inspiration.

sembles very closely the last part of the curves in figure 3, cannot be explained in this way. The cause of this increase in the nitrogen percentage must be either an increase in the total amount of nitrogen in the lung-bag system, or a diminution of the sum of the other gases, or a combination of both these factors. In other words, it must be due to the same factors which we considered responsible for the upward direction of the last part of the curves in figures 3 to 5.

Figure 7 represents curves from a normal individual (exper. 39), a patient with emphysema (no. 49) and a patient with mitral valvular disease (no. 51). The curves show the same main features. It is of

interest to note that mixture (indicated by an arrow) occurs later in the heart patient than in the other two subjects. The reason for this is presumably the smaller depth of the rebreathings of the heart patient (1.5 liters). The corresponding values for the other two patients are 4 to 3 liters. The lower the total volume is the lower is the course of the curve. The total lung volume of the normal subject was 5.9 liters, of the emphysematic 5.5 liters and of the heart patient 3.4 liters.

Analyses of samples drawn from bag and lungs in the same experiment. That our explanation of the results (and the form of the curves) is correct, can be given further support by determination of the nitrogen percentage in both the lungs and the bag after several respirations in the same experiment. This can be accomplished by using two sets of capillary lead tubes, one placed between the mouth and the 3-way stopcock, the other placed in the bag, which is a combination of the two previous procedures. Such an experiment is not an easy task and it requires considerable practice to be able to obtain a sufficient number of samples. Our intention was to take 16 samples, 8 of each kind during an experiment. A certain number, however, always went wrong. The cause of this was usually obstruction within the long lead tubes going from the short ones in the glass tube to the sampler. In other instances we failed in turning the stopcocks just at the right time. Three such experiments are recorded in table 4, and figure 8 gives two curves worked out on the basis of the figures in experiment 45. The results of the three experiments, however, being the same, these curves may typify what is going on in all. Either of the two correspond closely to the curves in figures 3 to 7. Taken together they show clearly the mechanism of mixing. Theoretically the two curves should meet when complete mixture is obtained and then continue parallel to the abscissa. However they do not meet. At the end of the first steep part of both curves the upper (alveolar) curve changes its direction before it reaches the lower curve, and both curves run from now on fairly parallel in a slight upward direction.

B. EXPERIMENTS WITH HYDROGEN. Hydrogen is used more generally than oxygen in determining lung volume. It is particularly suitable for that purpose for the following reasons: it does not play any rôle in metabolism, and its solubility in blood and in the moist film in the lungs is very small. It must always be washed by passing through solutions of potassium permanganate and tested for arsenic by Marsh's test. In tables 5 and 6 are reported a series of experiments showing the mechanism of mixing when hydrogen is used. The technique is,

TABLE 4

Determinations of nitrogen and carbon dioxide in samples drawn from the bag and from the very last part of the expiration without disturbing rebreathing
(See fig. 1)

EXPERIMENT NUMBER IN PROTOCOL	NUMBER OF RESPIRATION WHEN SAMPLE WAS DRAWN	ALVEOLAR AIR		GAS IN BAG		REMARKS
		Nitrogen	Carbon dioxide	Nitrogen	Carbon dioxide	
		per cent	*per cent*	*per cent*	*per cent*	
40	1½			52.64		Normal subject. Total lung volume 6 liters. Vital capacity 4.6 liters. Residual air 1.4 liters. Experiment started from maximum inspiration. Two liters of oxygen in bag at the beginning of the experiment. Depth of rebreathing about 4 liters. Respiration rate 7
	2½	63.74		58.49		
	3½	61.66		59.92		
	4½	62.90		60.16		
	5½					
	6½			61.60		
45	1½	64.88	4.39			Patient with emphysema of lungs and bronchial asthma. Total lung capacity 7 liters. Vital capacity 4.8 liters. Residual air 2.2 liters. Rebreathing started from maximum inspiration. Two liters oxygen in bag at the beginning of the experiment. Depth of rebreathing about 4 liters. Respiration rate 11
	3½			61.72	4.49	
	4½	62.46	4.87			
	5½	62.61	5.17	62.19	5.10	
	7½			62.72	5.75	
46	½	78.98	5.45			Patient with emphysema of lungs and bronchial asthma. Total lung volume 6.7 liters. Vital capacity 4.25 liters. Residual air 2.45 liters. Two liters oxygen in bag at the beginning of the experiment. Rebreathing started from maximum inspiration. Depth of rebreathing about 3.5 liters. Respiration rate about 13
	1½	63.42				
	2½	62.82				
	3½	63.14	5.62			
	4½	63.89		62.03	5.98	
	7½	64.03	7.24			
	8½			63.60	7.03	

as a whole, the same as in the oxygen experiments. A 5-liter rubber bag is used (fig. 2). At the beginning of the experiment 2 liters of gas are introduced into the bag. This gas contains 15 to 20 per cent hydrogen. In order to prevent the oxygen percentage from decreasing

too much during the experiment, the rest of the gas in the bag is either oxygen or a mixture of atmospheric air with oxygen. The gas analyses are done with a Krogh-Haldane apparatus. In table 5, 14 *different* experiments on 2 normal individuals are given. The first two series

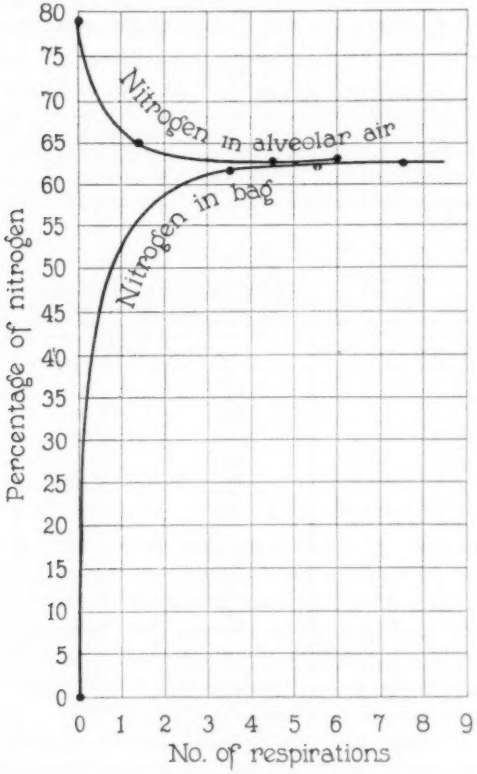

Fig. 8. Nitrogen values in samples drawn from bag and from alveolar air during the same experiment (exper. 45, table 4.)

of experiments (nos. 1a and 1b) are performed on one individual (the same subject as in exper. 39, table 3, where his lung volumes are given). The last series (no. 2) is performed on a normal individual whose lung volumes were not determined. All the experiments were started with the lungs in maximum inspiratory position. Consequently the

first respiration was an expiration into the bag, in which ½ a liter of hydrogen and (usually) 1½ liters of oxygen were introduced. After a certain number of respirations (½, 1½, 2½, and so on) the experiment was stopped and samples of alveolar air and bag gas were analyzed. The sample of alveolar air was collected from the mouthpiece into a non-evacuated sampler. In the 3 first experiments in series 1a, the

TABLE 5

Determination of hydrogen percentage in the bag and the alveolar air in 14 different experiments on two normal subjects. All the experiments started from maximum inspiration. After a varying number of rebreathings the experiment was stopped and samples drawn; 0.5 liter of hydrogen and 1.5 liters of oxygen in the bag at the beginning of the experiment. Depth of respirations 3 to 3.5 liters. Respiration rate 12 to 15.

SERIES	EXPERIMENT NUMBER	NUMBER OF REBREATHINGS OF THE EXPERIMENT	HYDROGEN IN ALVEOLAR AIR WHEN REBREATHING WAS FINISHED	HYDROGEN IN THE BAG WHEN REBREATHING WAS FINISHED
			per cent	*per cent*
1a	1	½	0.0	14.40
		1½	4.51	6.12
	2	2½	5.38	5.57
	3	3½	5.32	5.32
	4	7½	4.87	4.86
	5	8½	4.98	4.97
	6	9½	5.49	5.48
1b	7	3½	5.24	5.47
	8	5½	4.58	4.58
	9	7½	5.52	5.47
	10	10½	6.10	5.87
2	11	½	0.0	8.72
	12	1½	7.18	7.52
	13	2½	5.92	5.90
	14	3½	5.93	5.93

hydrogen percentage in the bag is higher than the percentage in the alveolar air. In experiment 4, which was stopped after 3½ respirations, the two percentages are equal. The same holds true in the next 3 experiments where the rebreathing was stopped after 7½, 8½ and 9½ respirations respectively. In these experiments the percentage of hydrogen in the alveolar air is a little higher than the percentage in the gas from the bag.

When the hydrogen percentage in the alveolar air exceeds or equals the hydrogen percentage in the bag, mixture must have taken place. This series therefore shows that under the conditions mentioned full mixture is obtained after $3\frac{1}{2}$ respirations. It is of interest that in an oxygen experiment on the same person, mixture also was obtained after $3\frac{1}{2}$ respirations (exper. 39, table 3). In the next series on the same individual mixture was not obtained after $3\frac{1}{2}$ respirations, whereas experiments stopped after $5\frac{1}{2}$, $7\frac{1}{2}$ and $10\frac{1}{2}$ respirations showed full mixture. This series shows more clearly than the first one that the hydrogen percentage in alveolar air has a tendency to exceed the percentage in the bag when the experiment is carried on longer than is necessary for the mixing (fig. 9). In the third series mixture was obtained after $2\frac{1}{2}$ respirations.

TABLE 6

Analyses of samples of air from the first and the very last part of the expiration. The samples are drawn into evacuated recipients. One single experiment

NUMBER OF RESPIRATIONS AT WHICH SAMPLE IS DRAWN	PERCENTAGE OF HYDROGEN IN THE FIRST PART OF AN EXPIRATION	PERCENTAGE OF HYDROGEN IN THE VERY LAST PART OF AN EXPIRATION
$1\frac{1}{2}$	4.62	3.90
$3\frac{1}{2}$	5.34	5.30
$5\frac{1}{2}$	5.44	5.48
$7\frac{1}{2}$	5.48	5.52

In table 6 the results of one single experiment are reported. Samples of air from the first part and the very last part of an expiration are drawn from the mouth piece into evacuated samplers, after $1\frac{1}{2}$, $3\frac{1}{2}$, $5\frac{1}{2}$ and $7\frac{1}{2}$ respirations, without disturbing the breathing. After $1\frac{1}{2}$ and $3\frac{1}{2}$ respirations, the hydrogen percentage in the first part of the expiratory air is higher than in the last part, indicating that mixture has not yet taken place. After $5\frac{1}{2}$ and $7\frac{1}{2}$ respirations, the opposite holds true. Mixture has therefore taken place between respiration $3\frac{1}{2}$ and $5\frac{1}{2}$. Although, *a priori*, it might be expected that full mixture would occur earlier with hydrogen than with oxygen because of the lower specific gravity of hydrogen, our results do not show any difference. From this, we draw the conclusion that the rules regarding mixture of hydrogen and oxygen (specific gravity 1 and 16) also can be used for carbon monoxide and nitrous oxide (specific gravity 14 and 22). The oxygen experiments show, as previously mentioned, the characteristic feature that the percentage of nitrogen in the lung air and in the bag increases after the mixture is complete. Furthermore the per-

centage in the lung air is always above that in the bag. Curves representing the nitrogen percentages in the two series of samples do not cross (fig. 8). This is due to factors which have nothing to do with the mechanism of mixture. In hydrogen experiments similar factors are encountered. In the first place, a small amount of hydrogen is absorbed by the blood and mucous membrane causing a decrease in hydrogen percentages of alveolar air: secondly, on account of the increasing carbon dioxide tension in the lungs, a smaller amount of carbon dioxide passes from the blood to the alveolar air. This fact

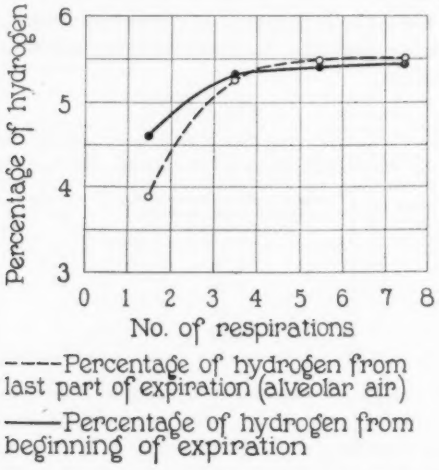
Percentage of hydrogen
6
5
4
3
0 1 2 3 4 5 6 7 8
No. of respirations
----Percentage of hydrogen from last part of expiration (alveolar air)
——Percentage of hydrogen from beginning of expiration

Fig. 9. Hydrogen values in samples drawn from the first part and the very last part of the expiratory phase during rebreathing. Experiment in table 6. Crossing of the two curves indicates that mixture has taken place. Normal subject.

tends to diminish the total volume of gas in the lungs and bag. If the oxygen percentage in bag is kept above normal during the experiment, the amount of oxygen absorbed by the blood will be greater than normal, a circumstance which may diminish the total volume of gas in the lungs and bag and cause an increase in hydrogen percentage. If, on the other hand, the oxygen percentage in the bag is low (which usually is avoided) the oxygen absorption will decrease. This might result in a tendency to increase the total volume of gas in the lungs and bag, and, as a further result, decrease the hydrogen percentage in the lung air. What the final result of the action of these different factors

will be, is difficult to predict. If the oxygen percentage in the bag is high (which has been the case in all our experiments) it is likely that the factors increasing the hydrogen percentage first in the alveolar air, and later on in the bag, will preponderate. The result of this will be that after full mixture has been obtained, the percentage of hydrogen in the lungs will exceed the percentage in the bag (fig. 9). This is seen in almost all of our experiments (table 5 and 6).

Our experiments confirm the conception that it is possible to mix air in the lungs with oxygen and hydrogen. In addition, the experiments show that under "natural" conditions mixture takes place between the second and fifth respiration. In oxygen experiments the mixture is complete when the nitrogen percentage in alveolar air is higher in the $n + 1^{th}$ respiration than in the n^{th}. And the mixture has taken place after the n^{th} if the nitrogen percentage in a sample drawn after this respiration is lower than that in a sample drawn after the $n - 1^{th}$ respiration. In order to determine this, it is necessary to analyze samples from at least 3 consecutive respirations. If we want to determine lung volume, for instance, the nitrogen percentage in alveolar air cannot be used because it is a little too high (usually from 1 to 3 per cent). The more correct figure will be the nitrogen percentage in air drawn from the bag after the n^{th} respiration.

If hydrogen is used in the bag in connection with sufficient oxygen, mixture is complete when a sample drawn from the bag after the $n + 1^{th}$ respiration shows a higher value than the n^{th}; and the mixture has taken place after the n^{th} respiration, if a sample drawn after this respiration shows a lower hydrogen percentage than a sample drawn after the $n + 1^{th}$. A simpler way is to find the first respiration after which the hydrogen percentage in the alveolar air is equal to or higher than the hydrogen percentage in the bag. If the oxygen percentage introduced into the bag is lower than that in the lungs, the mixture might be complete even if the percentage of hydrogen in the alveolar air stays below the percentage in the bag.

SUMMARY

1. Our experiments confirm the conception held by most investigators that it is possible to produce a mixture between air in the lungs and various gases and they show also that such a mixture is rather easily obtained. In a series of experiments on normal individuals, on patients with emphysema of the lungs, and on patients with heart disease, mixture could be obtained after from 2 to 5 *deep* respirations. In spite

of the difference between the specific gravity of oxygen and hydrogen, we were unable to detect any difference in the mixing rate of the two gases. We therefore draw the conclusion that our results with oxygen and hydrogen can be extended to nitrous oxide and carbon monoxide.

2. By drawing several samples of gas from the bag and from the alveolar air, during a mixing experiment, the mixing mechanism has been investigated. Several factors, though irrelevant as far as the mixing is concerned, may produce effects which give the impression that complete mixture is either impossible or difficult.

3. Procedures for determination of the time of full mixture have been devised. Full mixture is according to our definition present when the original difference between lung air and the gas in the bag (spirometer) has disappeared.

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STUDIES ON THE MIXTURE OF AIR IN THE LUNGS WITH VARIOUS GASES

II. The Quantitative Influence of Certain Factors in Producing a Full Mixture of Hydrogen with Air in the Lungs

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In a previous publication (1) it was shown that admixture of lung air with other gases (oxygen and hydrogen), under ordinary conditions, was usually obtained between the 2nd and 5th respiration. A procedure was given for determining whether proper admixture had been reached in our experiments with hydrogen and with oxygen, and it was stated that we thought it justifiable to extend the results of our research on oxygen and hydrogen also to hold true when nitrous oxide and carbon monoxide were used. It is, however, evident that it would be of importance if we could, in a given case, predict the conditions necessary for proper mixture.

We can, *a priori*, state a number of factors which influence the course of admixture. Such factors are:

Group A.
1. The nature of the gas with which the lung air is mixed.
2. The relative amount of the different gases in the spirometer or bag.
3. The amount of gas in the spirometer or bag.
4. The size of the dead space of the lungs and of the spirometer.
5. The rate of respiration.

Group B.
6. The number of respirations.
7. The depth of the respirations.
8. The amount of air left in the lungs (residual air plus more or less of the vital capacity).

If we knew the quantitative influence of all these eight factors on the course of admixture, we should theoretically at least be able to calculate what would happen at any given combination of these factors. In

order to obtain such knowledge, it seems necessary to examine the influence of variations of one factor when all other factors are kept constant. The problem at first seems almost an impossible task because of the very large number of variations which theoretically may take place. However, the character of the methods in which the mixing principle is used limits the variations of these factors to a much smaller number.

DISCUSSION OF INFLUENCE OF THE FIVE FACTORS ENUMERATED IN GROUP A. From what we already know it is evident that a quantitative study of the influence of variations in the factors enumerated in group A is of much less importance than a similar study of the factors in group B.

The reason for this is first that some of these factors may be varied outside the limits generally observed before the influence of such variations reaches a degree comparable to even the smallest variation in the number of respirations (namely one respiration); second, that other of these factors are of such a nature that one standard may be used in all the methods where admixture of lung air with other gas is needed. As to the nature of gases, only four kinds have so far been used, namely, oxygen, hydrogen, nitrous oxide and carbon monoxide.

The following experiments are performed on hydrogen. We believe, however, that the results can be extended to the other gases as well. As to technical details see paper I of this series. The percentage composition of the gases in the bag or spirometer influences by changing the alveolar air particularly the amount of nitrogen passing from the blood to the alveoli or the amount of oxygen or nitrogen passing into the blood. Large variations may therefore undoubtedly cause a small error in the final percentage of hydrogen (or nitrogen). In practice such large variations do not occur. If, for instance, hydrogen is used in determining lung volumes, the composition of the gas in the spirometer at the start usually is approximately 20 to 25 per cent hydrogen, the rest consists either of oxygen (as in our experiments) or of 30 to 40 per cent oxygen and the same amount of nitrogen. In a few of our experiments the percentage of hydrogen was smaller without any appreciable influence on the result.

If the oxygen method is used in determining lung volume, one usually starts with pure (or almost pure) oxygen in the bag. Through the above mentioned changes in the final composition of the alveolar air a small error in the figure for the lung volume may ensue, but no influence on the mixing process itself is noticed.

In none of the methods where the mixing principle is used is it necessary to introduce more than 3 liters of gas into the spirometer at the beginning of the experiment and in most instances 2 liters or even less are sufficient. This means that all or most of the gas in the spirometer can be drawn into the lungs during the inspiratory phase of the rebreathing. Only in determining the total capacity of the lungs is it necessary to have a considerable "residual" gas in the spirometer. The "residual" gas in this instance equals at least the amount of gas introduced into the spirometer at the beginning.

In almost all our experiments the amount of gas introduced into the spirometer or bag at the beginning has equaled the depth of the rebreathings, so that no "residual" gas has been present. This, of course, is the condition which is the most favorable for producing mixing. In a few experiments (table 2) a "residual" gas of 1 to 2 liters was present in the bag. It is possible that the process of mixing was a little delayed in these instances, the experiments are however too few to allow a final decision about this point. If a spirometer with a fast running propeller is used, the influence of 2 liters "residual" gas in the spirometer is undoubtedly very small.

The "dead space" of the system includes trachea, mouth and the tube going to bag or spirometer. In all our experiments this factor has been kept approximately constant. The tube has been 30 cm. long and 2 cm. wide. At the 3-way stopcock the passage was somewhat narrower being about one-half of the area of the tube.

The rate of respiration has varied somewhat. In most experiments it was around 10 to 15. A few experiments were performed in order to determine quantitatively the influence of the rate. We did not arrive at definite results. Our impression was that the rate may be varied considerably without any appreciable influence on the result.

EXPERIMENTS ON THE INFLUENCE OF THE THREE FACTORS ENUMERATED IN GROUP B. The three factors included in group B (the depth of respiration, the number of respirations, and the amount of air left in the lungs after the respiration) exert by far the largest influence on the course of the mixture. And in many instances where the mixing principle is used, the size of one or more of them is determined by the circumstances and not by the experimenter. The depth of the respiration cannot be increased above the vital capacity of the subject on whom the experiment is performed. The air left in the lungs after an expiration during the experiment can never be smaller than the residual air of the individual. The number of respirations which can be used

cannot be increased above a certain point, usually 3 to 9 according to the method of which the mixing process is a part. In determining the lung volume, for instance, it is possible to use about 7 to 9 or even more respirations if necessary. In determining the rate of circulation (Krogh and Lindhard's method) or the diffusion rate of the lungs (M. Krogh) it is probably inadvisable to use more than 3 to 4 respirations.

The influence of these 3 factors we have determined by varying one of them while the other two were kept constant.

In our experiments full mixture is considered present at the point at which the percentage of hydrogen in the alveolar air becomes larger than or equals the percentage in the bag or spirometer. In a previous publication, this procedure is described in detail. The hydrogen analyses were done by combustion in a Krogh-Haldane apparatus.

In the first two series of experiments, two normal, well-trained subjects were used. The residual air in the first subject was 2 liters and in the second 1.3 liters. A rubber bag containing a varying amount of gas (about 25 per cent hydrogen and 75 per cent oxygen) was used. The bag was tested to see that no hydrogen was lost by diffusion during the time of the longest experiment. In all but 6 out of the 43 experiments the amount of gas included in the bag did not exceed the vital capacity of the subject who was therefore able to inspire all the gas of the bag at each inspiration. In the last 6 experiments on the second individual the amount of gas in the bag was increased above the vital capacity of the subject. In these 6 experiments there would therefore be some gas left in the bag after the inspiration corresponding to the difference between the amount originally introduced into the bag and the depth of the rebreathing of the subject. After a certain number of respirations the hydrogen percentage was determined in the bag and in the alveolar air. The results of these analyses are given in columns 5 and 6 of tables 1 and 2.

In accordance with what has been shown before, a larger hydrogen percentage in the bag than in the alveoli is taken as a proof that admixture has not yet occurred; whereas full admixture was supposed to be present as soon as the alveolar hydrogen percentage equaled or exceeded the percentage in the bag. By varying the number of respirations, it was ascertained between which two respirations admixture occurred. In column 7, table 1, the difference between the hydrogen in the bag and in the alveolar air is given. In all the experiments where the value is negative, mixture has not occurred; whereas a positive value or zero indicates admixture. The change from negative to

positive in these differences indicates the point where the two curves in figure 9 in the first publication intersect.

In order to obtain a sample of alveolar air and of gas from the bag at the end of an experiment, we did not want to use evacuated samplers because it sometimes happened that our experiments went wrong on account of obstruction of the capillary lead tube through which the gas was drawn. For this reason we tried to separate the last part of the expired air from the first part by quickly turning the stopcock to connect with a long rubber tube into which the last 300 or 400 cc. were expired. At the end of the experiment a sample of alveolar air was then drawn from this rubber tube just outside the stopcock The results we obtained by this method proved to be very variable. We found in some instances that the hydrogen percentage was smaller in the alveolar air than in the bag, where according to previous experiments we should expect full admixture. And the difference between the two samples did not show any fixed relation. For this reason we ascribed these inconsistencies to the method of taking alveolar air. It is easy to see that if a rubber tube is not absolutely washed out, a certain amount of atmospheric air will remain and decrease the percentage of hydrogen in the expired air. We therefore made a change in the technique which at once gave perfectly uniform results. Just before the end of the final expiration the bag was divided into two unequal parts by a soft forceps with a clamp attached. See figure 2 in first paper of the series. From the larger part the sample of bag gas was taken and from the smaller part, just outside the stopcock, the sample of alveolar air was drawn. The forceps was applied very quickly immediately before the expiration was finished so that the very last part of the expired air was segregated in the small portion of the bag nearest the stopcock. On account of the elasticity of the rubber wall of the bag, this procedure did not prevent the completion of the respiration. For the same reason the sample could be drawn from that part of the bag without creating a negative pressure. The results obtained on the first subject are given in table 1. At a depth of respiration of 1 liter mixture was obtained after 6 respirations. If the depth was increased to 1.5 liters the number of respirations required for obtaining admixture was decreased to 5. At 2.5 and 3 liters depth, admixture was found after 3 respirations. In all these experiments the air left within the lungs equaled the residual air of the subject, that is, 2 liters. On the second subject, table 2, 23 experiments were performed and the results

agreed very closely with the results on the first subject. At a depth of 1 liter, 7 respirations were required in order to obtain admixture; 1.5 liters depth required 5 respirations, and 2 liters depth required 3 respirations. If the depth is increased to 2.5 liters admixture is obtained

TABLE 1

Normal subject. Effect on mixture of variations in depth and number of rebreathings. The amount of air in the lungs after the expiratory phase of rebreathing is kept constant and equals the residual air which is 2 liters. Bag experiments

DEPTH OF RESPIRATION	AMOUNT OF AIR INTRODUCED IN BAG IN LITERS	NUMBER OF RESPIRATIONS IN EXPERIMENT	APPROXIMATE DURATION OF EXPERIMENT IN SECONDS	HYDROGEN PERCENTAGE WHEN EXPERIMENT WAS FINISHED		DIFFERENCE BETWEEN HYDROGEN PERCENTAGE IN ALVEOLI AND BAG
				Bag a	Alveolar air b	b − a
liter	*liter*		*second*			
1.0	1.0	4	15	13.08	13.01	−0.07
1.0	1.0	5	20	11.25	11.22	−0.03
1.0	1.0	6	20	13.32	13.32	±0.00
1.0	1.0	7	20	9.62	9.68	+0.06
1.0	1.0	10	30	9.13	9.25	+0.12
1.5	1.5	2	10	10.53	10.07	−0.46
1.5	1.5	3	15	12.64	12.45	−0.19
1.5	1.5	4	20	12.64	12.62	−0.02
1.5	1.5	5	20	15.02	15.12	+0.10
2.0	2.0	2	10	12.32	12.26	−0.06
2.0	2.0	3	15	11.85	11.87	+0.02
2.0	2.0	4	15	12.23	12.37	+0.14
2.0	2.0	5	20	11.44	11.50	+0.05
2.5	2.5	2	10	10.68	10.51	−0.17
2.5	2.5	3	15	8.95	8.98	+0.03
2.5	2.5	4	20	14.09	14.15	+0.06
2.5	2.5	7	30	12.00	12.19	+0.19
3.0	3.0	2	10	16.39	16.00	−0.39
3.0	3.0	3	15	16.38	16.38	±0.00
3.0	3.0	4	15	15.37	15.42	+0.05

after 2 respirations. By further increasing the depth to 3 liters, admixture is not obtained before the end of the third respiration. In the last 6 experiments the amount of gas in the bag exceeded the vital capacity of the subject; consequently a certain amount of gas was left in the bag after maximum inspiration. If the amount of gas in the bag

was 4 liters, which would leave a residual gas in the bag of 1 liter, 4 respirations were necessary in order to obtain full mixture. If the amount was further increased, however, the number of respirations

TABLE 2

Normal subject. Effect on mixture of variations in depth and number of rebreathings. The amount of air in the lungs after the expiratory phase of rebreathing is (except in last six experiments) kept constant and equals the residual air which is 1.30 to 1.35 liters. Bag experiments

DEPTH OF RESPIRATION	AMOUNT OF AIR INTRODUCED IN BAG IN LITERS	NUMBER OF RESPIRATIONS IN EXPERIMENT	APPROXIMATE DURATION OF EXPERIMENT IN SECONDS	HYDROGEN PERCENTAGE WHEN EXPERIMENT WAS FINISHED		DIFFERENCE BETWEEN HYDROGEN PERCENTAGE IN ALVEOLI AND BAG
				Bag a	Alveolar air b	b − a
liter	*liter*		*second*			
1.0	1.0	6	20	10.73	10.52	−0.21
1.0	1.0	7	20	15.43	15.48	+0.05
1.5	1.5	3	15	9.42	9.33	−0.09
1.5	1.5	4	20	13.12	13.05	−0.07
1.5	1.5	5	20	13.68	13.78	+0.10
1.5	1.5	6	20	10.95	10.99	+0.04
2.0	2.0	2	10	12.37	11.94	−0.41
2.0	2.0	3	15	12.62	12.64	+0.02
2.0	2.0	4	20	12.66	12.78	+0.12
2.0	2.0	5	20	12.40	12.45	+0.05
2.5	2.5	1	—	10.53	10.06	−0.47
2.5	2.5	2	10	10.04	10.07	+0.03
2.5	2.5	3	15	11.02	11.04	+0.02
2.5	2.5	4	20	11.87	11.93	+0.06
3.0	3.0	2	10	11.43	11.32	−0.11
3.0	3.0	3	15	10.32	10.32	±0.00
3.0	3.0	4	20	11.49	11.54	+0.05
3.0	4.0	3	15	19.07	19.02	−0.05
3.0	4.0	3	15	16.13	16.00	−0.13
3.0	4.0	4	20	16.42	16.44	+0.02
3.0	5.0	2	10	14.82	14.76	−0.06
3.0	5.0	3	15	13.77	13.82	+0.05
3.0	5.0	4	20	14.22	14.30	+0.08

required went down to 3. These figures suggest that a "residual air" in the bag of more than 2 liters does not materially affect the mixture if the depth of the respirations is not too small.

We have not entered further into the question of the effect of variations in the "residual air" of the spirometer, because one usually can adapt the amount of gas introduced into the spirometer to the depth of the rebreathings. It is mainly in determining total lung volume that a certain "residual air" must necessarily be present.

It is of more importance to investigate the effect of variations in the amount of air left in the lungs after the expiratory phase of the rebreathing. The theoretical minimum for this air is the residual air of the subject. In practice, however, the amount of air left will usually always be somewhat greater, because the last part of the reserve air can be expired only with great effort and through a considerable prolongation of the expiratory phase. As a rule one will have to allow about half a liter more than the residual air. In spite of a difference of 0.7 liter in the residual air of the two subjects in tables 1 and 2, no marked difference is seen in the number of respirations required in order to obtain mixture. However, under pathological circumstances, the residual air may be increased above 2 liters and it might happen that such an increase would make it more difficult to obtain mixture.

Our first thought was to compare normal individuals with emphysematic patients. However, we gave that up thinking that better information could be obtained by using normal subjects in whom we varied the amount of air left in the lungs after the expiratory phase of the rebreathing. We proceeded as follows: A normal, well-trained subject with a residual air of 2 liters (see table 1) and a vital capacity of 5.85 liters, breathed through a 3-way stopcock connected with 2 Krogh spirometers A and B. After having filled his lungs with air (total volume 7.85 liters) a definite amount of air (v liters) was expired into spirometer A. In the lungs are now $7.85 - v$ liters. The stopcock is then quickly turned to connect with the other spirometer which contains hydrogen mixture in an amount corresponding to the depth of respiration during the following rebreathing. Spirometer B is therefore emptied (apart from the gas in the dead space) during the inspiratory phase. By means of special marks on the levers of the spirometers, the subject can control his expiration so that the same amount of air is left in the lungs after the expiratory phase, and so that all rebreathings become of equal depth. After a certain number of rebreathings the subject expires to his residual air, and a sample is quickly drawn from the mouthpiece during the very last part of the expiration. In order to make the time for drawing this sample very short strong suction was applied. In this way a sample could be drawn in 2 seconds. We

TABLE 3

Normal subject. Effect on mixture of variations in (1) depth of rebreathings; (2) in number of rebreathings, and in (3) amount of air left in the lungs after expiratory phase of rebreathings. Spirometer experiments

EXPERIMENT NUMBER	AMOUNT OF AIR LEFT IN LUNGS AFTER EXPIRATION	DEPTH OF RESPIRATION	NUMBER OF RESPIRATIONS	HYDROGEN IN SAMPLE FROM SPIROMETER a	HYDROGEN IN SAMPLE FROM ALVEOLAR AIR b	DIFFERENCE BETWEEN TWO SAMPLES b − a
	liter	*liter*		*per cent*	*per cent*	
1	6.35	1.5	5	9.00	8.64	−0.36
2	6.35	1.5	8	12.08	11.95	−0.13
3	6.35	1.5	12	11.78	11.70	−0.08
4	6.35	1.5	13	14.49	14.47	−0.02
5	6.35	1.5	14	10.39	10.42	+0.03
6	6.35	1.5	15	10.87	10.95	+0.09
7	5.85	1.5	10	12.71	12.64	−0.07
8	5.85	1.5	11	10.47	10.44	−0.03
9	5.85	1.5	12	9.91	9.87	−0.04
10	5.85	1.5	13	10.24	10.22	−0.02
11	5.85	1.5	14	7.12	7.10	−0.02
12	5.85	1.5	15	12.01	12.02	+0.01
13	5.85	2.0	5	9.31	9.00	−0.31
14	5.85	2.0	5	10.70	10.27	−0.43
15	5.85	2.0	6	13.52	13.44	−0.08
16	5.85	2.0	7	8.74	8.75	+0.01
17	5.85	2.0	8	8.99	9.00	+0.01
18	4.85	1.5	9	11.97	11.90	−0.07
19	4.85	1.5	10	6.32	6.36	+0.04
20	4.85	2.0	6	7.50	7.43	−0.07
21	4.85	2.0	7	9.28	9.29	+0.01
22	4.85	3.0	4	9.54	9.48	−0.06
23	4.85	3.0	5	11.06	11.00	−0.06
24	4.85	3.0	6	10.82	10.82	±0.00
25	3.85	1.5	5	15.25	14.90	−0.35
26	3.85	1.5	6	11.51	11.51	±0.00
27	3.85	1.5	7	16.01	16.03	+0.02
28	3.85	2.0	5	9.12	8.91	−0.21
29	3.85	2.0	6	13.15	13.17	+0.02
30	3.85	3.0	4	10.15	10.05	−0.10
31	3.85	3.0	5	10.60	10.60	±0.00
32	3.85	4.0	2	10.47	10.13	−0.34
33	3.85	4.0	3	12.94	12.91	−0.03
34	3.85	4.0	4	10.29	10.30	+0.01
35	3.85	4.0	5	16.52	16.52	±0.00

TABLE 3—*Concluded*

EXPERIMENT NUMBER	AMOUNT OF AIR LEFT IN LUNGS AFTER EXPIRATION	DEPTH OF RESPIRATION	NUMBER OF RESPIRATIONS	HYDROGEN IN SAMPLE FROM SPIROMETER a	HYDROGEN IN SAMPLE FROM ALVEOLAR AIR b	DIFFERENCE BETWEEN TWO SAMPLES b − a
	liter	*liter*		*per cent*	*per cent*	
36	2.85	1.5	5	19.87	19.57	−0.30
37	2.85	1.5	5	17.33	17.14	−0.19
38	2.85	1.5	6	12.74	12.79	+0.05
39	2.85	1.5	6	19.77	19.75	−0.02
40	2.85	1.5	7	14.68	14.66	−0.02
41	2.85	2.0	4	14.00	13.78	−0.22
42	2.85	2.0	5	9.55	9.56	+0.01
43	2.85	3.0	3	13.48	13.21	−0.27
44	2.85	4.0	4	16.84	16.80	−0.04
45	2.85	3.0	5	11.07	11.16	+0.09
46	2.85	4.0	2	13.19	12.94	−0.25
47	2.85	4.0	3	12.35	12.33	−0.02
48	2.85	4.0	4	10.47	10.48	+0.01
49	2.85	4.0	5	14.80	14.81	+0.01
50	2.85	5.0	2	12.01	11.82	−0.19
51	2.85	5.0	3	12.95	12.95	±0.00
52	2.85	5.0	4	11.80	11.85	+0.05
53	2.85	5.0	5	11.21	11.24	+0.03
54	2.00	1.0	8	14.15	14.00	−0.15
55	2.00	1.0	9	15.12	15.14	+0.02
56	2.00	1.0	10	18.58	18.66	+0.08
57	2.00	1.5	4	16.40	15.98	−0.42
58	2.00	1.5	5	15.99	15.96	−0.03
59	2.00	1.5	6	13.22	13.24	+0.02
60	2.00	2.0	3	14.06	13.98	−0.08
61	2.00	2.0	4	17.33	17.36	+0.03
62	2.00	3.0	2	14.40	14.16	−0.24
63	2.00	3.0	3	15.05	15.05	±0.00
64	2.00	4.0	2	14.37	14.15	−0.22
65	2.00	4.0	3	13.22	13.23	+0.01
66	2.00	5.0	2	11.97	11.84	−0.13
67	2.00	5.0	3	15.26	15.31	+0.05

preferred that way of drawing samples to the use of previously evacuated samplers. At the same time a sample of gas was drawn from the spirometer itself. A detailed review of the various experiments are probably unnecessary. The results are given in table 3 and are presented in the same way as those in tables 1 and 2. When the

percentage of hydrogen in the alveolar air exceeds or equals the percentage in the spirometer complete mixture is assumed to be present. The way we have varied the three factors: the amount of air left in the lungs after the expiratory phase of the rebreathing, the depth of respirations, and the number of rebreathings, is shown in table 3.

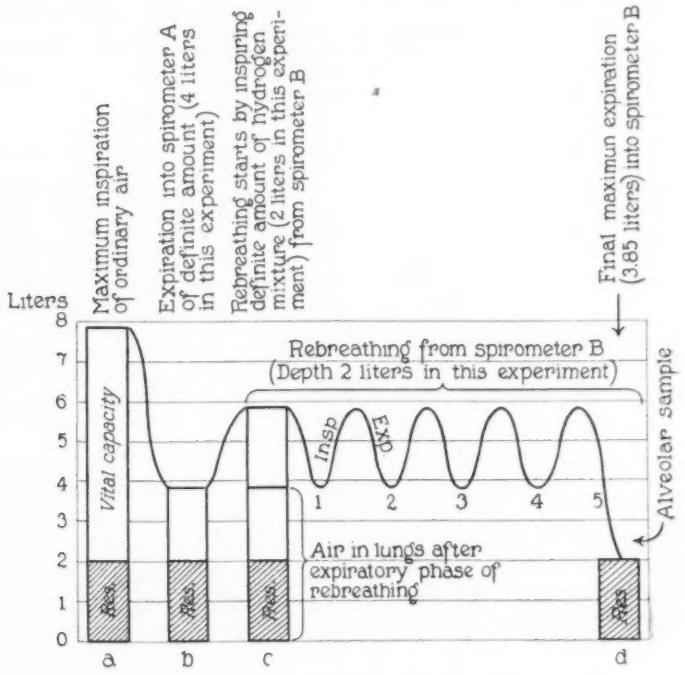

Fig. 1. Diagram illustrating the experimental conditions in table 3. This diagram refers to experiment 28. If one more rebreathing is added it would represent experiment 29. By varying each one of the different factors in turn the diagram may be brought to represent all the experiments.

In figure 1 we have shown in a graphic way the procedure in one of the experiments (no. 28 in table 3). The following experiment (no. 29) differs from no. 28 only in respect to the number of rebreathings. Any of the experiments could be presented in the same manner. In table 4 the main results of all the experiments are given. Table 5 gives the results in a still more compressed way.

TABLE 4

Relations between amount of air left in lungs after expiration. Depth of respiration and number of respirations in experiment showing full mixture of lung air with hydrogen in two normal individuals

AMOUNT OF AIR LEFT INSIDE LUNGS AFTER EXPIRATION (RESIDUAL AIR PLUS MORE OR LESS OF VITAL CAPACITY)	DEPTH OF RESPIRATIONS	NUMBER OF RESPIRATIONS SHOWING FULL MIXTURE
a. Rebreathing from a Krogh spirometer		
liter	*liter*	
6.35	1.5	14
5.85	1.5	13
	2.0	7
4.85	1.5	10
	2.0	7
	3.0	6
3.85	1.5	6
	2.0	6
	3.0	5
	4.0	4
2.85	1.5	6
	2.0	5
	3.0	5
	4.0	4
	5.0	3
2.00	1.0	9
	1.5	6
	2.0	4
	3.0	3
	4.0	3
	5.0	3
b. Rebreathing from a 5-liter rubber bag		
2.00	1.0	6
	1.5	5
	2.0	3
	2.5	3
	3.0	3
1.30	1.0	7
	1.5	5
	2.0	3
	2.5	3
	3.0	3

It is not difficult to apply our results to those methods, in which a complete mixture of lung air with other gas is a necessary condition. We deem it therefore unnecessary to enter into a discussion of the practical application of our results.

TABLE 5

Relations between depth of respirations, number of respirations, and air in lungs, securing complete mixture. Based on 109 rebreathing experiments with a hydrogen-oxygen mixture

		DEPTH OF RESPIRATION IN LITERS							
		1	1.5	2	2.5	3	4	5	
Amount of air left unexpired in lungs during mixing.	6.35		14						Number of rebreathings necessary to secure full mixture
	5.85		13	7					
	4.85		10	7		6			
	3.85		6	6		5	4		
	2.85		6	5		5	3	3	
	2.00	9 (6)*	6 (5)	4 (3)	(3)	3	3	3	
	1.30	(7)*	(5)	(3)	(3)	(3)			

* Figures in brackets refer to experiments performed with a rubber bag (see tables 1 and 2). Other figures refer to experiments performed with a Krogh spirometer (see table 3).

SUMMARY

A systematic study has been made of the quantitative influence of variations in the three main factors concerned in the production of mixture of air in the lungs with a hydrogen-oxygen mixture. These factors are (1) the amount of air in lungs after expiration, (2) the depth of rebreathing, and (3) the number of rebreathings. The main results, which cannot be expressed in a short summary are given in tables 4 and 5.

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THE INFLUENCE OF THE GONAD HORMONES ON THE SEMINAL VESICLES

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The function of the seminal vesicles has been variously interpreted. Some investigators maintain that the seminal vesicles are reservoirs for spermatozoa; others claim that they store the secretion formed by the prostate gland. That the seminal vesicles are secondary sex characters seems to be shown by their dependence upon the testis, castration inducing marked changes in size and secretory activity.

The work here reported was undertaken at the suggestion of Dr. C. R. Moore (10), with the view of determining the influence of the testis and ovaries on the seminal vesicles in the rat and the guinea pig.

The seminal vesicles open into the dorso-lateral part of the urethra, in the vicinity of the colliculus seminalis. They appear as blind sacs lying in the peritoneum between the rectum dorsally and the bladder ventrally. In the adult guinea pig, the seminal vesicles attain a length of approximately 12 cm. Each vesicle is constricted toward its inferior end to form the ductus excretorius which opens into the urethra. Distally and immediately adjoining these openings are the orifices of the vasa deferentia.

The walls of the seminal vesicles are made up of three layers of smooth muscle. The intermediate circular layer is always well developed. Within this circular layer there is a thinner longitudinal layer. Outside the circular layer, there is usually a thin longitudinal layer of muscle, but it is occasionally lacking. Covering the muscle wall is a thin serosa.

Within the muscularis a loose submucosa extends up into the longitudinal folds of the mucosa. The mucosa is made up of tall columnar eptihelial cells which contain demonstrable granules. The relatively small lumen is filled with a gray viscous fluid.

Leuckart (6) suggested that secretions of the seminal vesicles and the prostate were mixed to form a plug in the vagina, thus preventing the escape of the sperm, particularly in the guinea pig. Landwehr (5)

attributed the formation of the vaginal plug to the seminal vesicle secretion plus a ferment in the vagina. As copulation in the guinea pig takes place generally 2 to 4 hours after delivery, Landwehr thought the concentration of the vaginal ferments sufficient to produce the coagulum of the seminal vesicle secretion. Walker (16) reported that the secretion of the "coagulating gland," a small gland associated with the seminal vesicles containing a slightly viscous fluid, is responsible for the coagulation of the seminal vesicles' secretion in the vagina, and not the prostatic secretion or the ferments present in the vagina. Some investigators consider the seminal vesicles as reservoirs for spermatozoa. Pitha (11) took this position in spite of Leydig's (7) contention that the few spermatozoa found in the seminal vesicles are merely "occasional intruders." Thomas and Harrison (15), upon examination of the seminal vesicles of 52 human male cadavers, report the presence of spermatozoa in nearly all instances, except in cases of spermatocystitis. The presence of spermatozoa in the seminal vesicles after death convinced these men that the seminal vesicles serve as reservoirs during life.

THE ABSENCE OF SPERMATOZOA FROM THE SEMINAL VESICLES. In order to determine whether the spermatozoa enter the seminal vesicles, a series of twenty adult rats and twenty adult guinea pigs was killed and the contents of the seminal vesicles were studied immediately after death. A number of smears was made from each vesicle, as well as suspensions in physiological salt solution. In no case were any spermatozoa found in the seminal vesicles, but they were present in great numbers in the adjoining ampullae of the vas deferens and throughout the length of the latter.

Then, in order to see if the spermatozoa enter the seminal vesicles after death, ten rats were killed and placed in an incubator from 3 to 5 hours at body temperature. Smears and suspensions as above were made, without discovering a single spermatozoon in the vesicles.

In order to determine whether the spermatozoa enter the seminal vesicles at the time of copulation, a number of guinea pigs were placed separately with females; some of these were permitted to become sexually excited, but were not allowed to copulate, whereas others were permitted to copulate a varying number of times even to the point of fatigue. Some of these males were killed and the vesicles examined immediately, while others were killed later at varying intervals. In only one case, and this after several copulations were performed, was I able to find spermatozoa in the vesicles, and in this case there were but two.

From these results one may conclude that spermatozoa are rarely found in the seminal vesicles of the rat and guinea pig, and hence the vesicles cannot be properly termed reservoirs for the spermatozoa. The results support the view of Leydig who considered the spermatozoa in the seminal vesicles merely as "occasional intruders." The presence of spermatozoa in the seminal vesicles of human cadavers does not necessarily mean that they are normally there during life. It is possible that they enter the vesicles after death.

FORMATION OF THE SEMINAL VESICLE SECRETION IN THE YOUNG. *Influence of copulation.* There has been a lack of agreement among investigators as to whether the seminal vesicles actually produce an external secretion or merely store the secretion formed by the prostate. Leydig (7) thought the nature of the epithelium of the seminal vesicles sufficient reason for classifying them as secretory organs. Sedgwick and Minot (9) strongly emphasized the possibility of the prostate forming the secretion, and the storing of the secretion in the seminal vesicles. Akutsu (1) attributed a real secretory function to the vesicle epithelium. Zimmerman (18) conjectured that further research would link the formation of the secretion with the nuclei of the epithelial cells.

In order to determine at what age secretion appeared in the vesicle, a series of young guinea pigs was studied at various ages after birth. The epithelium of the seminal vesicles of guinea pigs one day old is transparent, the cells are columnar, the nuclei central, and the cell contents scarcely visible, with no granules in the tall narrow columnar epithelium. About the third day after birth the transparency of the epithelium has begun to disappear, due to the appearance of granular material within the cells. No secretion is seen within the lumen until about the seventh day when there appears a little fluid and a considerable number of extruded nuclei. Within the cells, between the nuclei and the lumen, many round vacuoles are present. At 15 days of age practically no nuclei are found in the lumen, and the epithelial cells are filled with granules.

The only histological change in the seminal vesicles of guinea pigs, as a result of sexual excitement, is a slight shifting of the cell nuclei toward the lumen of the vesicle. If copulations are permitted to the point of fatigue, a greater shifting of the nuclei toward the lumen occurs accompanied by an almost total disappearance of the secretion from the lumen. Four hours after excessive copulation in almost every cell a vacuole appears between the cell nucleus and the free border of the

cell. It is probable that these vacuoles are a phase in the formation of the secretion, since we find them in great numbers in the vesicles of very young guinea pigs, when the secretion is being formed, as well as after excessive copulation when all the secretion is expelled from the lumen of the vesicle.

THE INFLUENCE OF THE SEX GLANDS ON THE VESICLES. *a. The effect of castration.* Hendrich (3) found that the size and development of the seminal vesicles depended upon the presence of the testes. He reached this conclusion by observing that the early removal of the testes caused the seminal vesicles to remain small, or if the vesicles were already large, to atrophy. De Bonis (2) reported that in the guinea pig castration produced atrophy of the vesicles without entirely inhibiting the secretion. The injection of testicular extract produced no change in the vesicles.

To test the validity of the above work, fifteen rats were castrated at different ages and allowed to live varying lengths of time after the castration, before the vesicles were extirpated and studied. Castration of an adult rat causes atrophy of the seminal vesicles. The epithelium changes from the columnar to a flattened, cuboidal type. It appears as if the third of each cell bordering the lumen has simply vanished. It should be noted that it is this part of the columnar cell which is filled with granules in the normal vesicle. Some granules are found in the flattened type of epithelium 6 months after castration, suggesting that there is not a total inhibition, but a marked retardation of secretion. At this time a small amount of a thin milky fluid has replaced the very viscous normal secretion.

Following castration there is an apparent hypertrophy of the muscular layers of the vesicles. The muscle fibers appear to be forming in the reticular tissue of the folds which extend into the lumen of the vesicles. As a result, the bases of the folds are widened and the heights decreased by the increase of the reticular tissue within the folds.

When only one testis is removed, there is no change in the seminal vesicles. This fact shows that the mechanical factors in the operation are not responsible for the atrophy of the seminal vesicles.

b. Transplantation of the seminal vesicles. To determine with certainty whether the seminal vesicles formed a secretion of their own, a number of vesicles were transplanted to other parts of the body of the same and of different animals. In the case of the guinea pig, the vesicles were taken from an animal one day old. At this age there is no secretion in the lumen of the vesicles and no sign of granules in the cells.

When the vesicle, which had been grafted subcutaneously in a young male, was removed one month later, it was approximately four times its original size, filled with secretion, and appeared similar to a normal vesicle of the same age. Thus we see that an immature vesicle, transplanted before secretion is in evidence, increases in size and fills the lumen with secretion. This shows clearly that the vesicle is a secretory organ and not a mere reservoir for the prostatic secretion. The secretion must be a product of the epithelium.

In adult rats the distal ends of the seminal vesicles were extirpated, the secretion removed from the lumen, and the vesicle stumps fixed by suture to the peritoneal wall. Within one month the proximal ends of the transplanted vesicles became closed and the walls much distended with the newly formed secretion.

The seminal vesicles were taken from a young male rat and transplanted intraperitoneally in a spayed female. The testes from the young rat were transplanted subcutaneously in the same female. One month later the secretion in the lumen of the transplanted vesicle was similar to that found in the vesicles of castrated males. The epithelium, instead of the tall columnar, was somewhat flattened. In this case, the testis grafts had been absorbed. Two more vesicles from a rat 6 weeks of age were transplanted intraperitoneally in a castrated rat. Examination 3 weeks later revealed excellent vascularization and an apparent increase in size. Microscopic sections showed only a small amount of secretion present, and the epithelium had practically disappeared in some places, while in others it was very much flattened. That is, the tall columnar epithelium had undergone marked retrogressive changes.

It is therefore evident that the seminal vesicles will persist and secrete when transplanted to another part of the body of the same male or of another male. An immature vesicle can be transplanted to another male and develop secretory function.

The results from transplantation of the seminal vesicles into the same male or a different male with the gonads intact were definite. However, the few negative cases where transplantations were made into spayed females and castrated males do not permit of a definite conclusion. Since the latter grafts were well vascularized, one would be inclined to believe that the quantitative and qualitative change in the secretion and the atrophy of the epithelium was due to the absence of the testes.

c. Effect of ovarian grafts on the seminal vesicles. The question of antagonism of sex hormones has received considerable attention within the last few years. Steinach (14) maintains that sex hormones inhibit

heterologous secondary sex characters in the same species. That is, an ovary successfuly grafted into a male animal leads to the production of female characters and inhibits those structures that are specific for the male. He has maintained that a sex gland transplanted to an animal of the opposite sex will not persist unless the gonads of the host are first removed.

Such an antagonism has been disproved by Moore (10). This investigator has shown that an ovary may be transplanted into a male animal with one normal testis intact, the animal being a functional male, and the graft be recovered some months later. The ovarian tissue was healthy and very typical of the normal gland. Similarly testicular grafts into normal females persisted and were recovered some months later. The seminal vesicles of males bearing ovarian grafts were not studied microscopically by Moore, but it was noted that large ovarian transplants, persisting for a period of eight months " . . . have had no apparent inhibiting effect upon the penis, testis, sperm sacs (seminal vesicles), or psychical nature of the animal." If the ovary does exert an inhibitory influence upon the male characters we would expect the seminal vesicles to show some evidence of this.

The ovaries, taken from rats 10 to 15 days of age, were transplanted either subcutaneously or intraperitoneally, into eight normal males between 30 and 45 days of age. Two ovaries were placed in each male. The following table reveals the success of these transplantations.

TABLE 1

Rats—Grafts of ovaries into normal males

	SERIAL NUMBER	AGE OF ANIMAL AT GRAFTING	AGE OF GRAFT AT KILLING	NUMBER OF GRAFTS PERSISTING
		days		
Intraperitoneal grafts	R S 44	30	90	2
	R S 44-1	30	90	2
	R S 44-3	30	90	2
	R S 44-3a	30	90	2
Subcutaneous grafts	R S 45-C2	45	93	1
	R 5 Ov. G. 3	36	114	1
	R 1 Ov. G. 1	41	139	1
	R 1 Ov. G. 2	41	139	0

Thus it will be seen that ovarian grafts will grow in normal males for at least 3 to 5 months after transplantation. Histologically, all but one graft show characteristic or normal ovarian tissue. One large graft consisted of 450 to 500 sections when cut at 10μ. The Graafian follicles of various sizes are present in an entirely normal condition, and unquestionably the normal process of maturation is continuing. The material differs in no essentials from conditions described by Moore (10) and the reader is referred to this paper for further details.

The seminal vesicles in all the males carrying successful ovarian grafts were normal in size and appearance. Histologically, no changes could be detected.

In the eight ovarian grafts placed in the peritoneum, ova were present in large numbers in various stages of maturation. This is generally considered an index of the normal functional activity of an ovary.

The results attained with ovarian grafts placed subcutaneously in normal males with their gonads intact are less satisfactory as we notice the persistence of only one graft in each of three cases and total disappearance in one other case. The fact that some of the subcutaneous grafts did not persist may have been due to different factors, the chief of which was probably failure to secure proper vascularization. At least, one cannot claim that there is an antagonism between the testes and ovaries sufficient to inhibit growth, especially when oögenesis is going on in a normal manner at the end of 3 to 5 month periods.

The excised grafts were all cut at 10μ, and in some cases as many as 480 sections of ovarian tissue, with follicles containing ova, were cut from a single graft. Thus we see there was present sufficient ovarian tissue to effect changes, if ovaries and testes exerted antagonistic influences. In no case was there an apparent trace of sex gland antagonism, and consequently no changes produced in the seminal vesicles. This work confirms Moore's (10) findings that an ovary or ovaries may persist in the presence of two intact testes for at least 5 months, whether they be transplanted subcutaneously or intraperitoneally.

SUMMARY

1. The seminal vesicles in rats and guinea pigs are not reservoirs for spermatozoa or the prostate secretion although an occasional sperm may be found in the fluid.

2. Granules first appear in the cells of the seminal vesicles about the third day after birth; the secretory processes are well established at

twenty days. Vacuoles in the vicinity of the nuclei, soon after birth and after excessive copulation, may indicate secretory activity.

3. Transplantation of the seminal vesicles to other parts of the body does not interfere with the secretory activity of the vesicular epithelium.

4. Unilateral castration produces no effect on the seminal vesicles.

5. Bilateral castration, performed early, inhibits the growth of the vesicles; castration after the vesicles have developed causes atrophy (change from the columnar type of epithelium to the cuboidal type) but without the secretory process being totally inhibited.

6. In the process of degeneration, following castration, the muscle layers of the seminal vesicles are greatly increased.

7. The seminal vesicles are not affected by the presence of ovaries in normal males with intact testes, whether placed subcutaneously or intraperitoneally, for periods of 3 to 5 months.

I wish to acknowledge my indebtedness to Dr. C. R. Moore for his keen interest in this work and for his helpful criticisms, and to Dr. A. J. Carlson for his revision of the manuscript.

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THE BLOOD PRESSURES AND HEART RATE, IN GIRLS, DURING ADOLESCENCE

A Statistical Study of 1700 Cases

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To attempt to cite and comment upon all the investigative work done upon blood pressure in girls during the years of adolescence would be tedious and of little value for other than historical purposes. Then too, now that the Riva Rocci method has become almost universally adopted to the exclusion of the more complicated and often less accurate technique used with older forms of instruments, the former standards are of little value.

Of all the literature reviewed by the writer, there are possibly three papers which deserve recognition at the present time.

It is unfortunate that Wolfensohn-Kriss (1) who employed the von Basch as well as the Riva Rocci apparatus in a study of 350 school children, should have made use of the narrow cuff, because she treated her data carefully and her results show consistent variations. She concludes that, "Der Blutdruck steight mit zunehmen Alter, Körperlänge und Körpergewicht."

The paper on blood pressure in girls of early ages by Judson and Nicholson (2), published in 1914, is the best bit of investigation along this line which has come to the writer's attention. These authors used three methods of obtaining pressure; the auscultatory, a modified Erlanger recording method and a pith-ball oscillation apparatus. As noted in the discussion, the size of the cuff was adapted to the size of the arm. "In all cases we used the largest cuff available in proportion to the arm and a smaller cuff for comparative results." School children in recumbent posture were used throughout and over 1300 observations were made, systolic, diastolic and pulse pressures and pulse rate observed and charted. They conclude,

The systolic blood pressure varies with the increasing age of the child. Blood pressure changes with alterations in weight and, in children of the same age, varies according to height. The influence of sex is less than in the adult, with slightly higher readings in the males.

We find that the widest variations occur from the 10th to the 14th year, when there is a marked rise in the systolic pressure. The systolic pressure shows a slight but gradual rise from 3 to 10 years. From 10 to 14 years the increase is more abrupt, with a rapid elevation in the 14th year during adolescence. The systolic pressure varies from 91 mm. in the 4th year to 105.5 mm. in the 14th year.

In contrast to the systolic pressure, the diastolic remains at an almost uniform level.

Comparison of the results obtained by the auscultatory method, with those of the modified Erlanger method, shows remarkable uniformity in the readings. The auscultatory method requires no complicated apparatus, is easily carried out, and gives accurate results if the necessary precautions are taken to observe correct technique, especially as to the mode of application and width of the cuff.

While in the individual child the variations in the systolic pressure may be considerable, diastolic pressure remains fairly constant. The importance of determining the diastolic pressure in every case cannot be too strongly emphasized.

Very recently Alvarez (3) has published the records of systolic pressures taken on 4972 girls of university age, 16 to 26 years. Unfortunately the systolic pressure was the only record taken and although this was recorded with a form of the Riva Rocci instrument, the palpation method was followed. Arithmetical means were plotted against age and a gradual drop in height of pressure from 17 to 25 years and then a rise after that noted.

Little need be said about the change in heart rate with change in age, weight and height, since this has been well worked out years ago. Guy (4) gives a summary of the work which is comprehensive and is discussed later in this paper.

Vierordt (5) in 1855 also investigated this matter and recently Belot (6) on a small number of girls. Since arrhythmia is rather prevalent in children, Freiberger (7) considered arrhythmia in relation to pulse rate.

Since so much of the value of blood pressure findings depends upon the technique used as well as the care in using it, it seems advisable to go into a rather detailed consideration of the method employed in obtaining the data reported in this paper.

Blood pressure readings were taken on 837 girls in the Ithaca Public Schools whose ages varied from 9 to 20 years. Not only were the blood pressures taken but the heart rate, weight in pounds, height in inches, and age were recorded. It was also noted on each card whether or not the girl had reached the age of puberty, as indicated by the onset

of menstruation. Weights were taken with the clothing on and height with stockings on, or in case shoes were not removed, deduction was made for the height of the shoe heels.

The younger girls were brought into the medical office in small groups so that through the association of class mates the nervous element would be reduced to a minimum. Then, too, before the individual had her own pressure taken, she had been able to observe that the process had no unpleasant effects upon one of her friends. The school nurse and physician know these children personally so that it was felt that familiarity with surroundings practically eliminated emotional effects.

The blood pressures and pulse rate were taken with the child in the sitting posture and with a very few exceptions the cuff was placed upon the left arm. In certain cases of deformity the right arm could be employed to greater advantage, at the same time preventing embarrassment to the child. In all cases, of course, both the cuff and the stethoscope were applied over the bare arm.

Standard mercury manometers (Nicholson Princo) were used throughout, with the regulation wide cuff, i.e., 12 cm. width for the rubber bag and 13 cm. including the cloth covering. No difficulty was found in applying this width of cuff to a 9 year old girl.

The mercury was read to the nearest millimeter and at such a speed as to endeavor to combine accuracy of reading with avoidance of error due to congestion. The point taken for the reading of systolic pressure was the beginning of phase one and that for diastolic pressure at the beginning of phase four, since the auscultatory method of Korotkow was employed throughout this entire investigation.

Data on 847 young women students in Cornell University, between the ages of 16 and 26 years, were obtained from the office of the Medical Advisors to Women. The same methods were used for obtaining these data, that were employed by the writer and outlined in the preceding paragraphs. Here however the right and left arms were used about equally for obtaining the pressures.

Combining the data obtained from the school girls and university women, gives a total of 1684 cases.

There are, of course, but few points in the technique of even the simplest procedure, of any particular importance, but that have been attacked, disputed and often passed on to the clinician in a more or less debatable state still. So it is with blood pressure. And since this is true it may be well to state the opinions of other writers on the

various steps in the method of procedure as presented in the preceding paragraphs.

No attempt will be made to give the views of every investigator upon any one point. Such a task would be entirely without the scope of this paper.

In order to avoid confusion as much as possible, the order used in describing the technique will be followed in discussing it.

The objection will at once be raised that in the work with children of school age the emotional factor is bound to be great, especially with children in the lower grades. Every precaution was taken to reduce this, as indicated above, and it could be readily observed by the writer that the majority of even the smaller girls were only mildly interested rather than alarmed. O'Hare (8) has pointed out that excitement in patients may cause a rise of an average of 30 mm. systolic pressure and 12 mm. diastolic pressure. However he also found that when the pressures were taken at the beginning of the examination, the systolic was 21 mm. and the diastolic pressure 10 mm., on the average, higher than when the patient had been allowed to rest from 5 to 70 minutes. It will be noted that the emotional pressures are not markedly higher than would be found on first examination. Then too these were hypertension cases and according to the author their vascular regulating mechanism is more labile than normal.

The fact might be presented that many physicians urge taking the pressure several times and using the average. As Alvarez (3) points out, it is scientifically inaccurate to use an average of several readings on one individual, who may appear to the examiner to be more nervous than her neighbor, without doing the same thing for every child examined. The latter is manifestly impractical when a great number are to be examined. Then, too, the mere fact that the few cases of possible high readings from emotional children are averaged in with a great number of readings from unaffected individuals, would reduce such a possible error to insignificant proportions.

The blood pressures and pulse rate were taken with the individual in the sitting posture for several reasons. The most important reason was that the examiner felt that there would be less likelihood of excitement with the school girl in the usual resting position.

Stephens (9) found that the pressures averaged 3 to 4 mm. higher on the right than on the left arm. However, this difference is so small that it may safely be neglected.

It would seem hardly necessary to mention that the arm should be unclothed both over the site for the application of the cuff and that for the stethoscope bell. Oppenheimer and Bauchwitz (10) quote Gumprecht as saying early in the nineteenth century, "der bekleidete Arm zeigt einen höheren Druch als der nackte."

The use of mercury manometers throughout, rather than the aneroid type, precluded any error due to strain in the diaphragm and rendered frequent checking unnecessary.

While many seem to have found it difficult to use the standard width cuff on children, the writer experienced no difficulty with girls as young as 9 years. Judson and Nicholson (2), while working with blood pressures in children, studied the effect of varying the width of the cuff. "In all cases we used the largest cuff available in proportion to the arm and a smaller cuff for comparative results."

They found that in children from 4 to 8 years old, the circumference of the arm varied from 13 to 18 cm. so that a 9 cm. cuff gave as accurate results as an 11 or 13 cm. width. From 8 to 15 years the circumference of the arm was from 14 to 25 cm. At these ages more accurate results could be obtained with the 13 cm. cuff.

Michael (11) used a Riva Rocci instrument and 9 cm. cuff with children. She found a 10 cm. cuff gave an average reading 5 mm. lower, but was very hard to use on the small arms.

In adults, of course, the classical experiments of von Recklinghausen (12) in 1901 proved beyond question the necessity for the use of the wide cuff. Sahli (13), however, has raised objections to the necessity for the wide cuff.

Although some form of the Riva Rocci sphygmomanometer has been almost universally adopted for routine blood pressure examinations, in English-speaking countries at least, there has arisen some difference of opinion as to the relative accuracy of the original palpatory method as an index of the systolic pressure, and the more recent auscultatory method introduced by Korotkow (14) in 1905. Before considering the evidence on either side a decision as to what constitutes systolic pressure must be reached. If, as Kilgore (15) states, "the test of a good index is its ability to give constant results," then the conclusion of Kilgore, Berkley, Rowe and Stabler (16) is permissible: "So far as our experiments go, there is little to choose in point of definiteness between the common palpatory systolic index and the newer auscultatory criterion."

However, the desire of most investigators as well as clinicians is to know which method gives a result more closely approximating the actual maximal pressure in the arteries at the height of systole.

An excellent summary of the literature on the comparative readings by the palpatory or Strasburger method and the auscultatory or Korotkow method, given by Schrumpf and Zabel (17) in 1909, shows that the consensus of opinion places an auscultatory reading from 5 to 10 mm. higher for the systolic pressure. These authors themselves found the auscultatory readings higher. Kilgore (15) found 5 to 15 mm. difference in the two methods. This same difference was noted by Korotkow in his original communication.

The basis on which many of these investigators stated that the palpatory pressure was the normal and the auscultatory too high, was the results obtained by taking actual arterial pressures on men by placing a cannula in an artery. Many older experimenters tried this, Albert (18) and Faivre (19), for example.

But the comparison with the Riva Rocci readings was made by Müller and Blauel in 1907 (20). However, Wiggers (21) says, "Too great stress should not, however, be placed upon these results which were obtained, apparently, on three individuals by types of direct manometers, themselves not free from errors."

The auscultatory readings agree very closely with those of the recording types of sphygmomanometers and Erlanger (22), Fillner and Rudinger (23), and Wiggers, Eberly and Wenner (24) point out that these higher readings, when checked with animal experiments, are found to be correct. Since blood is shown by plethysmographic records to be forced under the cuff for a distinct interval of time before the first pulse beat is felt in the radial artery, thus allowing the mercury to drop from 5 to 15 mm. before a reading has been made, this method gives a reading obviously too low. Hill, Flack and Holtzmann (25) admit this when they conclude that, "In reading systolic pressures, there are two factors to take into account, 1. The actual maximal pressure of the cardiac output: 2. The conductance of the pressure wave by the artery"—and further state in the text of their paper, "We would point out here that the index used in these experiments, namely, cessation of flow, is not the same as the blockage of the pulse wave, the index used in living man."

The foregoing evidence would seem to indicate that if obtaining true systolic pressure be the aim of the examiner, the auscultatory method of Korotkow is preferable.

Not only has the auscultatory method for determining the point at which the systolic pressure should be read been questioned, but also this method for the determination of the diastolic pressure. The palpatory method is unquestionably eliminated here but the advocates of the oscillatory method have rather urgently contended for the superiority of their method in making diastolic readings. Then, too, among those who have accepted the auscultatory method as preferable there has been some discussion as to whether the onset of Korotkow's fourth or fifth phase should be the point at which a reading should be made.

When the auscultatory method is used in determining the systolic pressure, it would be clinically inexpedient to employ the oscillometer method for diastolic pressure even were it equally satisfactory.

Kilgore (26) found great difficulty in using the oscillometer method because of the large personal factor introduced especially in those forms without a graphic record. Zabel (27) in comparing the oscillometer and auscultation methods found that the fourth phase of the latter corresponded closely with the former. Warfield (28), working on dogs, came to much the same conclusion.

Ettinger (29), who first called attention to the so-called fourth phase as one to be considered in determining diastolic pressure, still considered the fifth phase the correct one to use.

An elaborate study made by Macwilliam and Melvin (30) resulted in the conclusion that a reading made at the beginning of the fourth auscultatory phase indicated the diastolic pressure and that this method was preferable to the oscillometer method for making the determination. Warfield (31) concludes, "We may affirm that there is both clinical and experimental evidence to prove that the point at which diastolic pressure should be read, when using the dial instrument, is at the point where the fling of the lever during the gradual lowering of pressure suddenly becomes less, or better, with the auscultatory method, where the clear sharp 3rd tone suddenly becomes dull."

The beginning of the fourth phase in the auscultatory method determining diastolic pressure seems to the writer to be so unquestionably established as to recommend its employment.

Having presented all the questions on technique which would seem to have been open to controversy, a consideration of the results obtained on adolescent girls will follow.

PART I: A STUDY OF ACTUAL ARITHMETICAL MEANS. In this first section of this paper, the writer has chosen to present the data in the

usual form, i.e., in the form of graphs, showing the arithmetical mean of the blood pressure or pulse rate plotted against age in years, height in inches or weight, in classes of 10 pounds each.

These arithmetical means were obtained by adding all the actual pressures for a given unit of age or height or class of 10 pounds and dividing this sum by the number of cases included.

Means were thus calculated for the school girl and college girl groups separately; for the menstruating and non-menstruating groups of the school girls and for the total group of girls who had reached puberty, as well as for the combined school and college groups.

It might be well to note here that throughout this paper when the writer refers to a group of girls as menstruating, the inference should be that the girls of this group have reached the age of puberty and have begun to menstruate regularly. It does not mean that the data were obtained during a menstrual period.

However, it was found that this method of treatment did not bring out group differences as well as the correlation method, presented in the second part of this paper. Therefore only the curves for the combined groups have been retained for discussion.

The figures used in plotting the curves seen in figures 1, 2 and 3 are shown in table 1, 2 and 3.

It will be noted in the curve in which systolic pressure is plotted against age (fig. 1, table 1) that there is a rapid rise in the pressure from 104.85 mm. at 9 years to 123.71 mm. at 14 years. The pressure remains at much the same level (123.29 mm.) through the next year. Then there is a rapid fall to 111.65 mm. at 18 years. From this age on the systolic pressure remains fairly constant, around 110 mm. up to the 26-year point. The average of all the ages is 113.17 mm.

The diastolic pressure rises evenly from 63.02 mm. at 9 years of age to 76.62 mm. at 14 years. It maintains about this level throughout the remaining years. The total average is 72.43 mm.

Pulse pressure, depending as it does upon the systolic and diastolic pressure values, starts at 42.29 mm. at 9 years, rises slowly to 49.38 mm. at 15 years and then quickly drops to 37.66 mm. at 18 years. It maintains this level rather consistently throughout the remaining 8 years. The average is 40.73 mm.

The pulse rate curve of course, instead of rising during the early years, drops rapidly from an average of 98.51 per minute at 9 years to 79.78 per minute at 18 years and then fluctuates little, up to 26 years. The average is 85.26 per minute.

In the height curves (fig. 2, table 2), plotted in inches, the systolic pressure rises rapidly and rather evenly from 98.00 mm. at 48 inches to 122.25 mm. at 70 inches.

The diastolic pressure increases from 66.50 mm. at 48 inches to 78.75 mm. at 70 inches. From this it can be readily understood that the pulse pressure will rise from 31.50 mm. to 43.50 mm. throughout the same range of height.

TABLE 1

Arithmetical means of systolic, diastolic and pulse pressure, and pulse rate and total cases for each unit of age

YEARS	CASES	ARITHMETICAL MEANS			
		Systolic pressure	Diastolic pressure	Pulse pressure	Pulse rate
9	65	104.85	63.02	42.29	98.51
10	77	108.69	65.57	42.99	99.19
11	122	109.48	64.93	44.74	93.42
12	92	114.16	68.67	45.60	92.67
13	100	118.55	71.45	47.10	92.11
14	90	123.71	76.62	45.98	90.87
15	116	123.29	73.91	49.38	87.53
16	75	118.92	73.77	45.15	84.84
17	121	115.41	75.59	39.84	81.75
18	175	111.65	74.01	37.66	79.78
19	193	111.76	75.01	36.75	79.34
20	181	109.93	74.13	35.69	78.39
21	137	109.50	73.21	35.99	79.73
22	62	110.50	75.08	35.42	79.59
23	35	105.91	72.86	33.17	80.60
24	16	110.69	76.31	34.38	75.00
25	13	112.62	75.15	37.46	76.58
26	14	111.43	72.50	38.93	81.00
Total average		113.17	72.43	40.73	85.26

The pulse rate dropped from 106.61 per minute at 50 inches to 80.50 per minute at 70 inches.

While readings were recorded for heights as small as 44 inches and as great as 71 inches, the number of cases included in the averages, excluded from this synopsis, was so small as to render the averages of these extremes of height of questionable value.

As noted above, the girls were grouped in classes in which the weights differ by ten pounds.

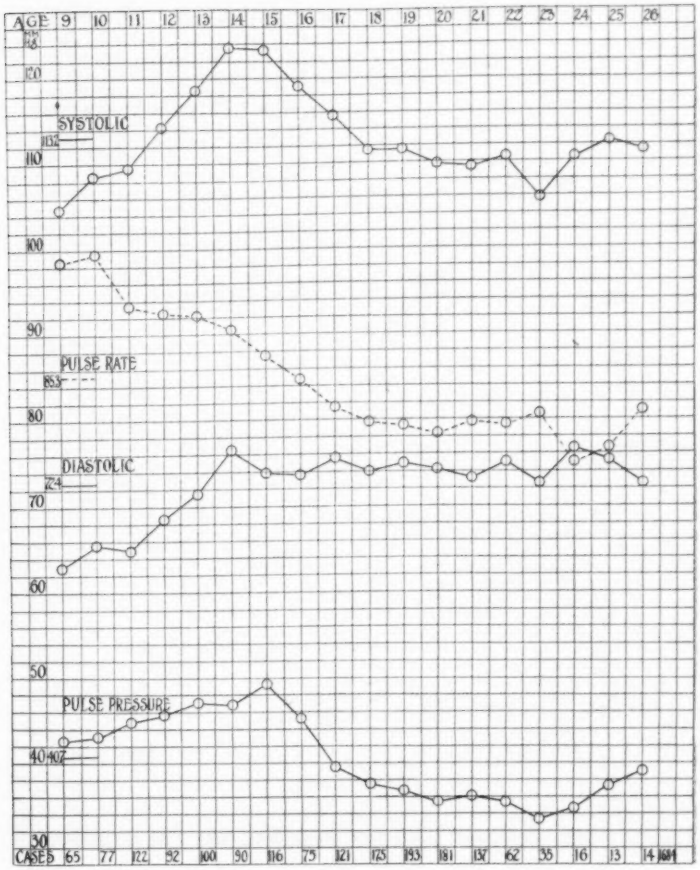

Fig. 1. Arithmetical means for ages 9 to 26 years plotted against systolic, diastolic and pulse pressure and pulse rate.

The systolic pressure (see fig. 3, table 3) rises rapidly from 104.18 mm. for the 51–60 pound class, up to 116.03 mm. for the 91–100 pound class. Then it runs along without much change until it reaches the 151–160 pound group (115.97 mm.). From this point on, the rise is abrupt up to 124.00 mm. in the 191–200 pound class.

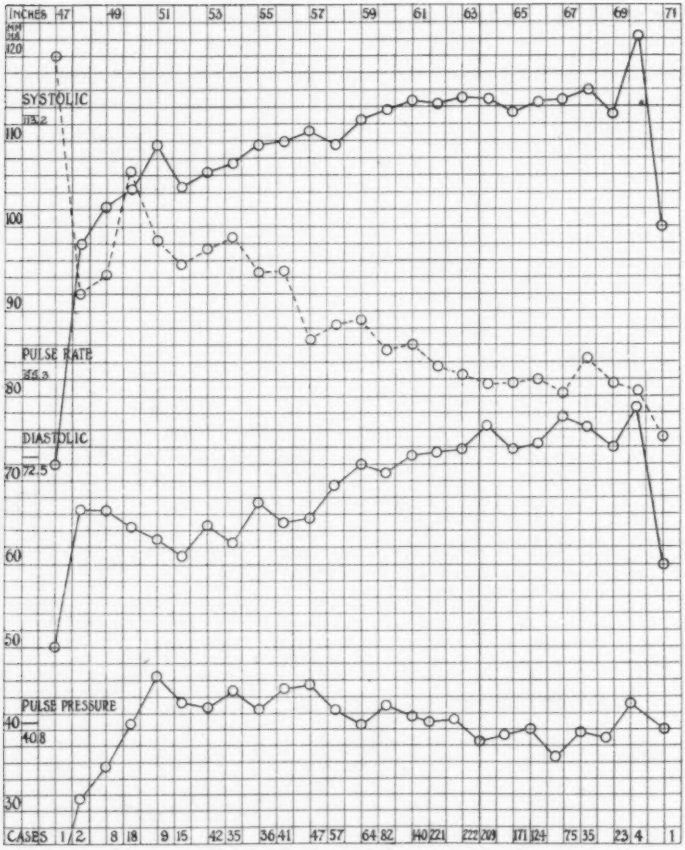

Fig. 2. Arithmetical means for height in inches, plotted against systolic, diastolic and pulse pressure and pulse rate.

The diastolic pressure shows a gradual rise from 61.49 mm. in the 51–60 pounds class to 87.50 mm. in the 191–200 pound group. The pulse pressure is bound to show irregularities following the relation between the irregularity of the systolic pressure curve and the regularity of the diastolic pressure curve.

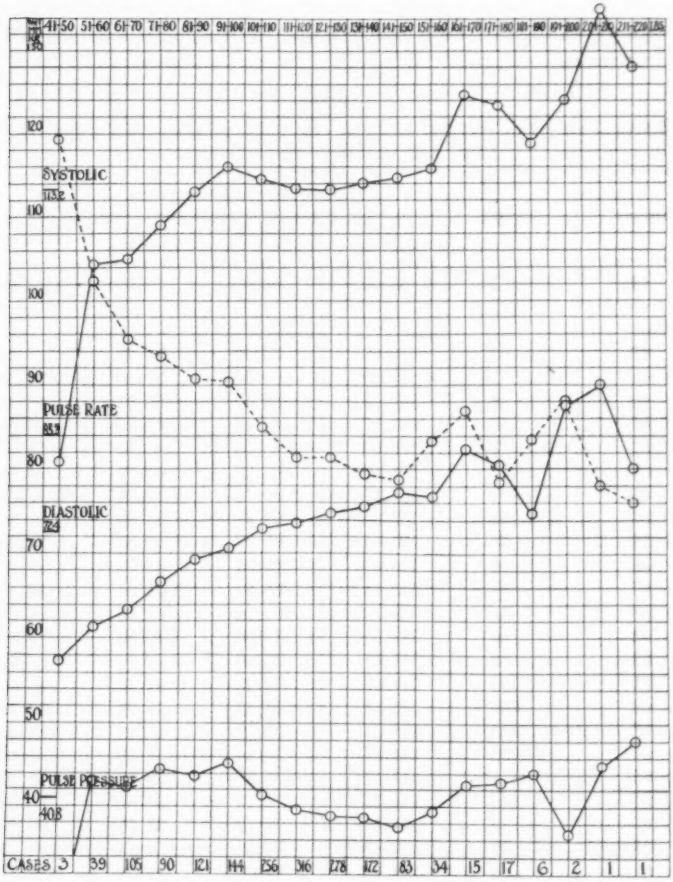

Fig. 3. Arithmetical means for weight in pounds, plotted against systolic, diastolic and pulse pressure and pulse rate.

The pulse curve drops from 102.15 per minute in the 51–60 pound class to 85.08 at the 101–110 pound class. From this point it continues somewhat unevenly with little change in general level throughout the remainder of the group.

In the case of these weight curves also, a group at the beginning and two groups at the end were omitted, due to deriving the averages from too few cases.

TABLE 2

Arithmetical means of systolic, diastolic and pulse pressures and pulse rate and total cases for each unit of height

INCHES	CASES	ARITHMETICAL MEANS			
		Systolic pressure	Diastolic pressure	Pulse pressure	Pulse rate
44	1	84.00	63.00	21.00	103.00
45					
46					
47	1	72.00	50.00	22.00	120.00
48	2	98.00	66.50	31.50	92.00
49	8	102.12	66.50	35.63	94.38
50	18	104.17	64.44	40.83	106.61
51	9	109.56	63.00	46.56	98.22
52	15	104.40	61.07	43.33	95.60
53	42	106.19	64.74	42.88	97.62
54	35	107.34	62.37	44.69	98.66
55	36	109.44	67.33	42.39	94.44
56	41	110.00	65.05	44.95	94.70
57	47	111.11	65.51	45.66	86.74
58	57	109.75	69.26	42.25	88.58
59	64	112.55	72.03	40.52	88.95
60	82	113.91	70.96	42.94	85.37
61	140	114.85	72.93	41.89	86.08
62	221	114.11	73.14	40.96	83.59
63	222	114.96	73.79	41.23	82.57
64	209	114.69	76.34	38.36	81.68
65	171	113.13	73.82	39.25	81.69
66	124	114.25	74.23	40.02	82.08
67	75	114.49	77.76	36.76	80.19
68	35	116.03	76.20	39.83	84.21
69	23	113.13	74.09	39.04	81.77
70	4	122.25	78.75	43.50	80.50
71	1	100.00	60.00	40.00	75.00
	1,683				

In all cases there was a gradual rise of diastolic pressure. That is, diastolic pressure increases gradually with an increase in age, height and weight. This presents the most uncomplicated of all the pressure curves and therefore would seem to be far more desirable as a standard for blood pressure than either systolic or pulse pressure.

Judson and Nicholson (2) are practically the only investigators who have reported diastolic pressures taken on children. The writer heartily endorses their statement that, "While in the individual child the variations in the systolic pressure may be considerable, diastolic pressure remains fairly constant. The importance of determining the diastolic pressure in every case cannot be too strongly emphasized."

TABLE 3

Arithmetical means of systolic, diastolic and pulse pressures and pulse rate and total cases for each group of 10 pounds of weight

POUNDS	CASES	ARITHMETICAL MEANS			
		Systolic pressure	Diastolic pressure	Pulse pressure	Pulse rate
41–50	3	80.67	57.67	23.00	119.67
51–60	39	104.18	61.49	42.95	102.15
61–70	105	104.94	63.27	42.06	95.56
71–80	90	108.91	66.51	44.37	93.56
81–90	121	113.06	69.12	43.93	90.90
91–100	144	116.03	70.55	45.33	90.13
101–110	256	114.39	73.12	41.32	85.08
111–120	316	113.57	73.91	39.72	81.32
121–130	278	113.33	74.42	38.84	81.29
131–140	172	114.06	75.55	38.40	79.43
141–150	83	114.78	77.23	37.55	78.65
151–160	34	115.97	76.71	39.26	83.66
161–170	15	124.60	82.27	42.33	86.69
171–180	17	123.29	80.41	42.88	78.24
181–190	6	118.83	74.83	44.00	83.67
191–200	2	124.00	87.50	36.50	88.00
201–210	1	135.00	90.00	45.00	78.00
211–220	1	128.00	80.00	48.00	76.00

However their statement, "In contrast with the systolic pressure, the diastolic remains at an almost uniform level," is not borne out by the results presented in this paper.

It may be well to point out here the fact that in this climate practically all girls have reached puberty as indicated by the onset of menstruation, by the time they reach their 14th birthday. The following table was compiled from the data on which this paper is based.

The systolic pressure curve for age rises rapidly until the onset of puberty. Then as the metabolic disturbances incident to the onset of catamenia tend to become less noticeable, in two or three years, the

systolic pressure curve falls until a point is reached from which there is little change throughout the remaining years considered in this paper.

Up to this time the majority of investigators have considered girls of ages either up to the approximate age of puberty or worked with adults alone. Hence the complete curve has not been followed through by the same individual, to the writer's knowledge.

In the case of the former group of investigators, the consensus of opinion has been for many years that blood pressure, meaning systolic pressure, increases with increase in age.

Tavaststjerna (32) in 1909 concluded that up to 15 years of age, in girls, there is a gradual rise of systolic pressure and from that point on it remains at much the same level. He further notes that Finnish girls with whom he worked reached puberty at 15 to 16 years of age.

TABLE 4

AGE	NUMBER MENSTRUATING	TOTAL NUMBER SCHOOL GIRLS
9	0	65
10	1	77
11	4	122
12	22	92
13	58	100
14	81	90
15	108	116
16	64	65

Wolfensohn Kriss (1) in 1910 concluded, "Daraus dürfen wir schliessen, das der Blutdruck bis zum Eintritt der Pubertat seine Hohe erreicht und dort eine constante Grosse aufweist."

Judson and Nicholson (2) sum up the whole subject in this statement, "We find that the widest variations occur from the 10th to the 14th year, when there is a marked rise in the systolic pressure. The systolic pressure shows a slight but gradual rise from 3 to 10 years. From 10 to 14 years, the increase is more abrupt, with a rapid elevation in the 14th year during adolescence."

In considering the peculiar curve for the systolic pressure when plotted against weight (fig. 3), if reference be made to table 5, the matter will be somewhat simplified. It will be noted that were the non-menstruating school girls eliminated here, the first abrupt rise from the 51–60 pound class to the 91–100 pound group would not appear in the curve. The averages would have been on much the

same level from the 51–60 pound class to the 151–160 pound group. The last abrupt rise is unique. That is, there would appear to be a direct relation between increase of systolic pressure in women and increase in weight after reaching the weight of about 150 pounds.

The first quick rise in the curve indicates that weight has a very close relation to systolic pressure in girls who have not yet reached the age of puberty.

TABLE 5

Arithmetical means of systolic pressure, with a separate group for non-menstruating, menstruating and total girls, and number of cases for each group of ten pounds

WEIGHT IN POUNDS	SYSTOLIC PRESSURE					
	Menstruating girls		Non-menstruating girls		Total girls	
	Arithmetical means	Cases	Arithmetical means	Cases	Arithmetical means	Cases
41–50			80.67	3	80.67	3
51–60	115.00	2	103.59	37	104.18	39
61–70	115.00	1	104.85	104	104.94	105
71–80	109.63	8	108.84	82	108.91	90
81–90	113.00	47	113.09	74	113.06	121
91–100	113.91	97	120.40	47	116.03	144
101–110	113.76	228	119.46	28	114.39	256
111–120	113.22	306	124.20	10	113.57	316
121–130	113.28	276	120.00	2	113.33	278
131–140	114.06	172			114.06	172
141–150	114.65	82	126.00	1	114.78	83
151–160	115.33	33	137.00	1	115.97	34
161–170	124.60	15			124.60	15
171–180	123.29	17			123.29	17
181–190	118.83	6			118.83	6
191–200	124.00	2			124.00	2
201–210	135.00	1			135.00	1
211–220	128.00	1			128.00	1

Practically all workers with systolic pressure in children have found that this pressure increased with an increase in weight. However, none has pointed out that in the case of girls, this increase is limited to the non-menstrual group.

Tavaststjerna (32) divided his adult individuals into two groups, one heavier than the average and the other lighter than the average. The systolic pressure for the heavy group was 5 to 15 mm. higher than that for the lighter one. However he concludes, "das Verhältniss

zwischen Körpergewicht und maximalem Blutdruck sehr inkonstant . . . . Hieraus folgt, das wir nicht erwarten dürfen, bei schwereren Individuen in der Regel einem verhältnismässig hohen maximalen Blutdruck zu begegnen."

It has been well established that there is an increase of systolic pressure with height during childhood. But it has not before been carefully pointed out that there is a gradual increase in systolic pressure with height in girls who come within the 9 to 26 year limits. This curve does not seem to show any marked fluctuation. Hence it is obvious that height is a much better standard for systolic pressure than either age or weight.

Pulse rate has been under discussion for so long a time, that there is little to be gained by further consideration here.

In the age curve, the pulse rate drops rapidly until the girl has recovered from the disturbances occurring at puberty. Then it remains rather constant throughout the remaining years considered.

CONCLUSIONS: 1. There is an increase of systolic pressure with increase of age until puberty. Allowing two or three years for these disturbances to abate and a concomitant slow drop in pressure, the systolic pressure varies little up to 26 years of age.

2. In non-menstruating girls there is an increase of systolic pressure with increase in weight. In the case of girls who have passed puberty there is little change in systolic pressure as weight increases, up to about 150 pounds. From this point on there seems to be a rapid increase of pressure with added weight.

3. There is a slow, gradual increase of systolic pressure with increase of height. This is so constant that height rather than age is to be recommended as a standard in determining the "normal" pressure for girls from 9 to 26 years of age.

4. Diastolic pressure increases gradually and evenly with increase in weight, height or age. It shows little or no disturbance due to puberty. It would seem to afford a decidedly less fluctuating index of vascular system condition than the much employed systolic pressure.

PART II: A STUDY OF THE CONSTANTS OBTAINED THROUGH AN APPLICATION OF STATISTICAL METHODS TO THE DATA. It will be noted, when considering the literature presented by others who have worked with blood pressure in normal individuals, that there has been a tendency to obtain the *arithmetical mean* of all the data for each unit of age, weight or height as the case might be, and from that, plot a curve to indicate the average change in blood pressure, with change in age, etc. For

the purpose of comparison with other work and for those who prefer results in this form, this method has been followed by the writer, in the first section of this paper.

Tavaststjerna (32) and Alvarez (3) have gone a step farther than this by classifying a large number of cases in a table with one variable, e.g., age along one axis and another variable, e.g., systolic pressure along the other. This presents to the eye the distribution of the data with reference to both variables.

The next logical step would be to apply statistical methods to obtain by mathematical means, a numerical expression which would indicate the degree to which a variation in the one character, e.g., age, tended to be followed by a variation in the other, e.g., systolic blood pressure. Such a numerical expression is found in the *correlation coefficient.*

By employing the correlation coefficient, it may be accurately determined, if the data represent a large number of individuals, whether the changes in blood pressure follow more closely change in age, weight or height.

The writer, using the data of 1700 cases of blood pressures and pulse rate, in girls between the ages of 9 and 26 years of age, constructed correlation tables and calculated the correlation coefficients for the characters, age in years, weight in pounds and height in inches, correlated with systolic, diastolic and pulse pressures and heart rate. Not only was this done, using the total number of cases but separate tables were made in the same manner for the non-menstruating and menstruating groups of school girls as well as for the total group of school girls. The data for the college girls were also worked out in separate tables. Then tables made for the total number of menstruating girls, of both school and college groups.

This paper will not permit a discussion of the mathematical basis of the correlation coefficient and related statistical constants. For this the reader is referred to works by Eugene Davenport (33), C. B. Davenport (34), Yule (35) or any other good work on statistical methods for the treatment of biological data.

The correlation coefficient is indicated by the letter r. This is calculated from the formula:

$$r = \frac{\frac{\Sigma P}{n} - (c_1 c_2)}{\sigma_1 \sigma_2}$$

n = the total number of cases studied or the total frequency.

If an increase of one character, e.g., age, is always followed by a corresponding and proportional increase in a related character, e.g., systolic pressure, the correlation is perfect, i.e., $r = +1.0$. If on the other hand an increase in one character is always followed by a corresponding and proportional decrease in a related character, the correlation is negative, i.e., $r = -1.0$. But if the characters are absolutely indifferent, one to the other, there is no correlation and $r = 0$.

To determine the degree of exactness of the correlation coefficient, r, once it has been calculated, the probable error, Er, is also calculated from the formula:

$$E_r = \pm \frac{0.6745}{\sqrt{n}} (1 - r^2)$$

If r is not three or four times greater than the probable error, it is not regarded as significant. M_1 and M_2 are the means or averages for the two characters used and c_1 and c_2 are the corrections for the means assumed, when the calculations were begun.

$$c = \frac{\Sigma f D}{n}$$

$$M = G \pm c \qquad E_m = \pm \frac{0.6745}{\sqrt{n}} \sigma$$

G = Assumed mean.

σ_1 and σ_2 are the standard deviations and are an expression of the tendency of the character to vary from its mean.

$$\sigma = \sqrt{\frac{\Sigma f (D)^2}{n} - c^2}$$

when f indicates the frequency and D is the deviation from the assumed mean:

$$E_\sigma = \pm \frac{0.6745}{\sqrt{2n}} \sigma$$

When there was occasion to determine the difference between two correlation coefficients the probable error for the difference was determined by the formula

$$P.E._D = \pm \sqrt{(E_{r_1})^2 + (E_{r_2})^2}$$

The correlation coefficients with their calculated probable errors will be found in the summary of constants in tables 6 and 7. The probable error for any single determination of blood pressure was found by means of the formula: $P.E._{s.o.} = \pm 0.6745\sigma$. A summary of the probable errors for single determinations will be found in table 11.

First the results obtained from a study of these correlation tables will be presented and then these results will be briefly discussed.

The greatest correlation of the systolic pressure series appears to exist between this pressure and weight in pounds, height in inches and age in years in this order, in the case of non-menstruating girls. These are of school, rather than college age, of course. The correlation coefficient for weight is $+0.493 \pm 0.026$; that for height $+0.413 \pm 0.028$ and that for age $+0.344 \pm 0.030$.

In the case of the menstruating school girls, the correlation is in all cases much less than in the non-menstruating. However, here too the greatest correlation is with weight, $+0.299 \pm 0.029$; the next with height, $+0.206 \pm 0.031$, and the least with age, where there is almost no significant correlation, $+0.071 \pm 0.032$.

It must be remembered that significant correlation exists only where the coefficient is at least three or four times greater than its probable error. Hence this eliminates the last coefficient mentioned.

An interesting feature of these data is discovered when the menstruating and non-menstruating school girl populations are combined. Considering the combined data, with regard to age, it will be noticed that the coefficient is $+0.412 \pm 0.019$, a figure about equaling the sum of the correlation coefficients for the menstruating and non-menstruating populations. If however weight is considered, the coefficient for the combined table is $+0.516 \pm 0.017$. This is much less than the sum of the two separate coefficients $+0.493 \pm 0.026$ and $+0.299 \pm 0.029$. The coefficient for the combined height data is $+0.475 \pm 0.018$, while the separate coefficients are $+0.413 \pm 0.028$ and $+0.206 \pm 0.031$.

This unexpected variation of the correlation coefficient for the total school girl population, without regard to either the sum or difference of the coefficients for the menstruating and non-menstruating groups of data, points to the seriousness involved in endeavoring to gain information as to the correlation between two variables, where there is a third factor introduced.

The unreliability of a single coefficient for two groups of this nature may be clearly seen by considering briefly two distribution tables. In table 8 the distribution is shown when pulse pressure of menstruat-

TABLE 6

Biometrical constants, with their probable errors, for systolic and diastolic blood pressures, correlated with age, height and weight

		r	E_r	M_1	E_{M_1}	σ_1	E_{σ_1}	M_2	E_{M_2}	σ_2	E_{σ_2}
						Systolic pressure					
	Correlated with age in years										
High school	Non-menstruating	+0.344	±0.030	11.031	±0.049	1.441	±0.035	110.235	±0.454	13.350	±0.320
	Menstruating	+0.071	±0.032	15.188	±0.060	1.875	±0.043	121.760	±0.419	13.100	±0.301
	Total	+0.412	±0.019	13.256	±0.061	2.673	±0.043	116.405	±0.331	14.410	±0.231
College	Total	+0.004	±0.023	19.861	±0.045	1.965	±0.031	108.150	±0.250	10.865	±0.174
Total	Menstruating	−0.349	±0.016	18.242	±0.056	2.946	±0.038	112.860	±0.254	13.360	±0.254
	Menstruating and non-menstruating	−0.108	±0.016	16.576	±0.065	4.049	±0.049	112.255	±0.214	13.405	±0.16
	Correlated with weight in pounds										
High school	Non-menstruating	+0.493	±0.026	78.840	±0.564	16.600	±0.398	110.235	±0.454	13.350	±0.320
	Menstruating	+0.299	±0.029	113.450	±0.636	19.860	±0.457	121.760	±0.419	13.100	±0.301
	Total	+0.516	±0.017	97.360	±0.581	25.240	±0.404	116.405	±0.331	14.410	±0.231
College	Total	+0.234	±0.022	124.170	±0.408	17.750	±0.284	108.150	±0.250	10.865	±0.174
Total	Menstruating	+0.082	±0.019	120.450	±0.365	19.190	±0.249	112.860	±0.254	13.360	±0.174
	Menstruating and non-menstruating	+0.177	±0.016	110.830	±0.409	25.580	±0.307	112.255	±0.214	13.405	±0.161
	Correlated with height in inches										
High school	Non-menstruating	+0.413	±0.028	56.692	±0.138	4.059	±0.097	110.235	±0.454	13.350	±0.320

	Menstruating	+0.206	±0.031	62.567	±0.091	2.849	±0.066	121.760	±0.419	13.100	±0.301
	Total	+0.475	±0.018	59.841	±0.104	4.529	±0.072	116.405	±0.331	14.410	±0.231
College	Total	+0.081	±0.023	63.775	±0.055	2.384	±0.038	108.150	±0.250	10.865	±0.174
Total	Menstruating	+0.009	±0.019	63.357	±0.050	2.625	±0.034	112.860	±0.254	13.360	±0.174
	Menstruating and non-menstruating	+0.152	±0.016	61.816	±0.066	4.123	±0.049	112.255	±0.214	13.405	±0.161

Diastolic pressure

	Correlated with age in years										
High school	Non-menstruating	+0.314	±0.031	11.031	±0.049	1.441	±0.035	66.045	±0.330	9.695	±0.233
	Menstruating	+0.168	±0.031	15.188	±0.060	1.875	±0.043	74.765	±0.326	10.200	±0.235
	Total	+0.439	±0.019	13.256	±0.061	2.673	±0.043	70.710	±0.250	10.870	±0.174
College	Total	+0.007	±0.023	19.861	±0.045	1.965	±0.031	72.395	±0.235	10.225	±0.164
Total	Menstruating	−0.043	±0.019	18.242	±0.056	2.946	±0.038	73.230	±0.195	10.255	±0.133
	Menstruating and non-menstruating	+0.215	±0.015	16.576	±0.065	4.049	±0.049	71.580	±0.169	10.545	±0.127
	Correlated with weight in pounds										
High school	Non-menstruating	+0.358	±0.030	78.840	±0.564	16.600	±0.398	66.045	±0.330	9.695	±0.233
	Menstruating	+0.326	±0.029	113.450	±0.636	19.860	±0.457	74.765	±0.326	10.200	±0.235
	Total	+0.500	±0.017	97.360	±0.581	25.240	±0.404	70.710	±0.250	10.870	±0.174
College	Total	+0.150	±0.022	124.170	±0.408	17.750	±0.284	72.395	±0.235	10.225	±0.164

TABLE 6—*Concluded*

		r	E_r	M_1	E_{M_1}	σ_1	E_{σ_1}	M_2	E_{M_2}	σ_2	E_{σ_2}
Diastolic pressure—Concluded											
Total	Menstruating	+0.177	±0.018	120.450	±0.365	19.190	±0.249	73.230	±0.195	10.255	±0.133
	Menstruating and non-menstruating	+0.346	±0.014	110.830	±0.409	25.580	±0.307	71.580	±0.169	10.545	±0.127
Correlated with height in inches											
High school	Non-menstruating	+0.341	±0.030	56.692	±0.138	4.059	±0.097	66.045	±0.330	9.695	±0.233
	Menstruating	+0.189	±0.031	62.567	±0.091	2.849	±0.066	74.765	±0.326	10.200	±0.235
	Total	+0.444	±0.018	59.841	±0.104	4.529	±0.072	70.710	±0.250	10.870	±0.174
College	Total	+0.068	±0.023	63.775	±0.055	2.384	±0.038	72.395	±0.235	10.225	±0.164
Total	Menstruating	+0.086	±0.019	63.357	±0.050	2.625	±0.034	73.230	±0.195	10.255	±0.133
	Menstruating and non-menstruating	+0.311	±0.014	61.816	±0.066	4.123	±0.049	71.580	±0.169	10.545	±0.127

ing school girls is correlated with age. The coefficient for this group is -0.063 ± 0.032; that for college girls, all of whom menstruate, is -0.021 ± 0.023; while the coefficient for the combined groups, is -0.415 ± 0.016. A glance at this table will reveal the fact that the increase in the coefficient for the combined groups over the sum of the two coefficients for the two groups taken separately, is due to the fact that the moving of the second group, college girls, toward the "y" axis has aligned it with the first, so that the general line of correlation is more perfect than that of either group alone. If now, reference is made to table 9, it will be noted that in this case this moving of one of the groups of the combination has resulted in a coefficient for the total which is far less than the sum of the two individual coefficients, i.e., the general line of correlation of each has been broken rather than reinforced by the shifting of the second group.

In girls of college age, 16 to 26, the greatest correlation is between the systolic pressure and weight also. It is $+0.234 \pm 0.022$. That for height is still less, $+0.081 \pm 0.023$, and that for age, $+0.004 \pm 0.023$. This last is insignificant.

Alvarez (3) published a correlation table of systolic pressures with age, in 4972 girls between 16 to 26 years. No correlation coefficient was calculated. So the writer calculated this, $r = -0.052 \pm 0.010$. This of course is not a significant correlation and thus checks very closely with that obtained for the data of college women from 16 to 26 years, presented here.

Since correlation of weight and systolic pressure is rather pronounced in menstruating school girls, ($+0.299 \pm 0.029$) and also in college girls ($+0.234 \pm 0.022$) it would at once be suggested that since both of these groups are menstruating, they might well be combined into one large table. If this is done, the combined correlation is far less than either of the others ($+0.082 \pm 0.019$).

The explanation for this was indicated in one of the previous paragraphs, i.e., there is some third factor involved which has resulted in making this a combination of two differing populations. The third factor in this case seems to be the metabolic disturbances due to puberty, persisting in the school girls. At just what age all traces of this have disappeared is not apparent from the present data. But it is reasonable to suppose that while a disturbance of this sort may persist in many of the menstruating school girls, it has probably almost entirely disappeared in the majority of college girls. That is, in one case there is a group of girls with a heightened metabolism to consider.

TABLE 7

Biometrical constants, with their probable errors, for pulse pressure and heart rate, correlated with age, height and weight

		r	E_r	M_1	E_{M_1}	σ_1	$E\sigma_1$	M_2	E_{M_2}	σ_2	$E\sigma_2$
Pulse pressure											
Correlated with age in years											
High school	Non-menstruating	+0.144	±0.033	11.031	±0.049	1.441	±0.035	44.235	±0.396	11.650	±0.280
	Menstruating	−0.063	±0.032	15.188	±0.060	1.875	±0.043	47.040	±0.412	12.870	±0.296
	Total	+0.097	±0.023	13.256	±0.061	2.673	±0.043	45.735	±0.285	12.400	±0.198
College	Total	−0.021	±0.023	19.861	±0.045	1.965	±0.031	34.440	±0.178	7.725	±0.124
Total	Menstruating	−0.415	±0.016	18.242	±0.056	2.946	±0.038	38.805	±0.219	11.500	±0.150
	Menstruating and non-menstruating	−0.361	±0.014	16.576	±0.065	4.049	±0.049	40.060	±0.188	11.760	±0.141
Correlated with weight in pounds											
High school	Non-menstruating	+0.224	±0.032	78.840	±0.564	16.600	±0.398	44.235	±0.396	11.650	±0.280
	Menstruating	+0.012	±0.032	113.450	±0.636	19.860	±0.457	47.040	±0.412	12.870	±0.296
	Total	+0.159	±0.022	97.360	±0.581	25.240	±0.404	45.735	±0.285	12.400	±0.198
College	Total	+0.116	±0.023	124.170	±0.408	17.750	±0.284	34.440	±0.178	7.725	±0.124
Total	Menstruating	−0.084	±0.019	120.450	±0.365	19.190	±0.249	38.805	±0.219	11.500	±0.150
	Menstruating and non-menstruating	−0.143	±0.016	110.830	±0.409	25.580	±0.307	40.060	±0.188	11.760	±0.141
Correlated with height in inches											
High school	Non-menstruating	+0.168	±0.033	56.692	±0.138	4.059	±0.097	44.235	±0.396	11.650	±0.280
	Menstruating	+0.051	±0.032	62.567	±0.091	2.849	±0.066	47.040	±0.412	12.870	±0.296
	Total	+0.156	±0.022	59.841	±0.104	4.529	±0.072	45.735	±0.285	12.400	±0.198
College	Total	+0.026	±0.023	63.775	±0.055	2.384	±0.038	34.440	±0.178	7.725	±0.124
Total	Menstruating	−0.082	±0.019	63.357	±0.050	2.625	±0.034	38.805	±0.219	11.500	±0.150
	Menstruating and non-menstruating	−0.133	±0.016	61.816	±0.066	4.123	±0.049	40.060	±0.188	11.760	±0.141

	ating	−0.133	±0.016	61.816	±0.066	4.123	±0.049	40.060	±0.188	11.760	±0.141

Pulse rate

	Correlated with age in years										
High School	Non-menstruating	−0.170	±0.033	11.031	±0.049	1.443	±0.035	95.190	±0.495	14.570	±0.350
	Menstruating	−0.315	±0.029	15.186	±0.060	1.877	±0.043	87.085	±0.443	13.840	±0.318
	Total	−0.365	±0.020	13.255	±0.062	2.674	±0.045	90.850	±0.339	14.750	±0.251
College	Total	−0.012	±0.024	19.982	±0.046	1.915	±0.033	79.215	±0.203	8.445	±0.144
Total	Menstruating	−0.347	±0.017	18.219	±0.057	2.999	±0.040	82.095	±0.216	11.390	±0.159
	Menstruating and non-menstruating	−0.492	±0.013	16.491	±0.070	4.095	±0.049	85.255	±0.229	13.455	±0.161
	Correlated with weight in pounds										
High school	Non-menstruating	−0.163	±0.033	78.750	±0.562	16.530	±0.397	95.190	±0.495	14.570	±0.350
	Menstruating	−0.125	±0.031	113.440	±0.636	19.880	±0.457	86.980	±0.447	13.970	±0.321
	Total	−0.285	±0.021	97.320	±0.581	25.260	±0.429	90.795	±0.341	14.830	±0.252
College	Total	−0.060	±0.024	124.210	±0.424	17.660	±0.300	79.215	±0.203	8.445	±0.144
Total	Menstruating	−0.172	±0.018	120.270	±0.365	19.210	±0.269	82.060	±0.217	11.430	±0.160
	Menstruating and non-menstruating	−0.443	±0.014	110.250	±0.437	25.720	±0.309	85.225	±0.229	13.485	±0.162
	Correlated with height in inches										
High school	Non-menstruating	−0.186	±0.033	56.688	±0.138	4.063	±0.098	95.190	±0.495	14.570	±0.350
	Menstruating	−0.029	±0.032	62.573	±0.092	2.877	±0.066	86.980	±0.447	13.970	±0.321
	Total	−0.257	±0.021	59.837	±0.104	4.543	±0.077	90.795	±0.341	14.830	±0.252
College	Total	+0.032	±0.024	63.814	±0.058	2.401	±0.041	79.215	±0.203	8.445	±0.144
Total	Menstruating	−0.067	±0.019	63.342	±0.050	2.645	±0.037	82.060	±0.217	11.430	±0.160
	Menstruating and non-menstruating	−0.360	±0.015	61.750	±0.071	4.175	±0.050	85.225	±0.229	13.485	±0.162

TABLE 8

Distribution tables to show how the total correlation coefficient may be increased by combining two populations, each with a lower correlation coefficient

Menstruating school girls, indicated in bold type in this table. $r = -0.063 \pm 0.032$.

College girls, indicated in light type in this table. $r = -0.021 \pm 0.023$.

Total menstruating girls. $r = -0.415 \pm 0.016$.

	6 10	11 15	16 20	21 25	26 30	31 35	36 40	41 45	46 50	51 55	56 60	61 65	66 70	71 75	76 80	81 85	86 90	91 95
10													1					
11						2		1		1								
12					1	1	4	5	3	3	3	1	1					
13			1	2	3	5	6	5	13	3	8	6	5					
14				1	5	11	13	13	10	5	13	4	3	2	1			
15			2	3	9	7	9	13	17	16	8	5	11	3	4	1		
16				2	1	7	10	12	9	7	10	3	1	1		1		
				1	2	4	3											
17			2	8	4	6	12	10	7	10	2	2	1		1			
					5	19	18	9	5									
18	1		3	1	3	4	4	4	5	4	3	2						1
				11	23	39	42	12	4	6	2							
19			5		2	2	5	1	2	4	2		1					
				15	33	46	39	19	10	5	1	1						
20			4	19	37	1	36	20	9	2	2	1	1					
						47												
21			1	7	31	44	30	13	6	5								
22			1	3	14	15	24	4		1								
23			3	4	10	5	8	3	2									
24			1	1	5	3	2	4										
25				1	2	6	1	2				1						
26			1	1	1	1	3	3	2									

When grouped together these people have a good correlation of weight and systolic pressure. And there is another group, the college girls, in which metabolism has dropped to a lower level. When grouped, these two have a good weight-pressure correlation. However, when the two groups are combined, the correlation has almost entirely disappeared.

That there is a difference between menstruating school girls and menstruating college girls is obvious from the following table (table 10). In this the correlation ratio of the college girls has been subtracted from that of the menstruating school girls throughout the whole series of blood pressure and pulse rate correlations presented in this paper.

TABLE 9

Distribution tables to show how the total correlation coefficient may be decreased by combining two populations, each with a higher correlation coefficient

Menstruating school girls, systolic pressure according to weight (indicated in bold type in this table). $r = +0.299 \pm 0.029$.

College girls (indicated in light type in this table). $r = +0.234 \pm 0.022$.

Total menstruating girls. $r = +0.082 \pm 0.019$.

	66 70	71 75	76 80	81 85	86 90	91 95	96 100	101 105	106 110	111 115	116 120	121 125	126 130	131 135	136 140	141 145	146 150	151 155	156 160	161 165	166 170	171 175
51 60								1				1										
61 70										1												
71 80						1 /	/			2	3											
81 90					1 3	1 3	1 2	8 2	2	5	8	2	3	3	1	1	1					
91 100				/	1 3	1 12	2 3	3 6	7 3	13 4	9 /	14 /	4 /	6	1	2						
101 110	/	2	/	7	14	16	4 15	8 29	10 11	13 15	17 4	14 5	14 2	15	6	2	2	1				
111 120			3	3	13	4 40	5 31	6 55	15 24	11 25	15 13	8 6	2	15	3	4	1	2			1	1
121 130				5	1 7	1 29	2 30	6 45	13 32	9 34	14 13	6 5	1	8 /	3 /	4 /	2	1				
131 140	/	2		2	6	18	23	1 24	7 18	7 24	3 9	3 7	1	4	1	4 /	2	1	1			
141 150				1	3	10	1 7	1 15	10	5 8	2 7	1 2	4 /	1	2	1						
151 160					1	3		11	6	5	1 /	/	2	/	/							
161 170						1		1		2	2 2	2 1	1	/	1	1		1				
171 180						1	2		2	3	2	1 /		1 /	1	1	1					
181 190									1	1 3		1										
191 200										1		/										
201 210													/									
211 220													/									

There is, with two exceptions, better correlation in the school girls. In the case of the two exceptions the difference is so small as to be negligible.

An analysis of the foregoing statement leads to the conclusion that in the consideration of the school girl and college girl blood pressure and pulse rate data, by means of the correlation coefficients, it is permissible to use only the following groupings; non-menstruating school girls, menstruating school girls and college girls. Any other combinations such as total school girl data, i.e., non-menstruating and menstruating; total menstruating, i.e., school girls menstruating and college girls or total data, i.e., total college and total school girls, are not

allowable since, in these cases, as previously mentioned, there is always likely to be a disturbance of the true value of r, due to the introduction of a factor other than the two which are being correlated.

The remainder of the discussion of the results of this series will therefore be confined to the consideration only of the legitimate coefficients of correlation.

In the case of the diastolic pressure, as in the systolic, the greatest correlation seems to exist between weight in pounds and diastolic

TABLE 10

Table showing difference between correlation coefficients of menstruating school girls and college girls

	MENSTRUATING SCHOOL GIRLS		COLLEGE GIRLS		DIFFERENCE	
	r	E_r	r_1	E_{r_1}	$r-r_1$	$E_r-E_{r_1}$
Correlation of						
Systolic pressure with:						
Age in years	+0.071	±0.032	+0.004	±0.023	+0.067	±0.039
Weight in pounds	+0.299	±0.029	+0.234	±0.022	+0.065	±0.036
Height in inches	+0.206	±0.031	+0.081	±0.023	+0.125	±0.039
Diastolic pressure with:						
Age in years	+0.168	±0.031	+0.007	±0.023	+0.161	±0.039
Weight in pounds	+0.326	±0.029	+0.150	±0.022	+0.176	±0.036
Height in inches	+0.189	±0.031	+0.068	±0.023	+0.121	±0.039
Pulse pressure with:						
Age in years	−0.063	±0.032	−0.021	±0.023	−0.042	±0.039
Weight in pounds	+0.012	±0.032	+0.116	±0.023	−0.104	±0.039
Height in inches	+0.051	±0.032	+0.026	±0.023	+0.025	±0.039
Pulse rate with:						
Age in years	−0.315	±0.029	−0.012	±0.024	−0.303	±0.037
Weight in pounds	−0.125	±0.031	−0.060	±0.024	−0.065	±0.039
Height in inches	−0.029	±0.032	+0.032	±0.024	−0.061	±0.040

pressure. Here also the non-menstruating school girls lead with a coefficient of $+0.358 \pm 0.030$. Height is next highly correlated, with diastolic pressure, $r = +0.341 \pm 0.030$ and then age, where $r = +0.314 \pm 0.031$.

The menstruating school girls show correlation in the same order, although with slightly lower coefficients; weight, $r = +0.326 \pm 0.029$; height, $r = +0.189 \pm 0.031$ and age, $r = +0.168 \pm 0.031$.

In the college girl group, the tendency is to follow the weight, height, age order but here as was the case with systolic pressure, the coefficients

are decidedly lower than in either of the school girl groups. Correlated with weight, $r = +0.150 \pm 0.022$; height, $r = +0.068 \pm 0.023$ and age, $r = +0.007 \pm 0.023$. This last is of course not statistically significant.

TABLE 11

Probable errors for single observations. These errors are expressed in millimeters, of mercury, of pressure. Of course all of these errors are + or −.

		SYSTOLIC PRESSURE	DIASTOLIC PRESSURE	PULSE PRESSURE	PULSE RATE
	Age				
High school	Non-menstruating	9.007	6.539	7.858	9.827
	Menstruating	8.836	6.880	8.681	9.335
	Total	9.720	7.332	8.364	9.949
College		7.328	6.897	5.211	5.696
Total	Menstruating	9.011	6.897	7.757	7.683
	Total	9.042	7.114	7.932	9.075
	Weight				
High school	Non-menstruating	9.007	6.539	7.858	9.827
	Menstruating	8.836	6.880	8.681	9.423
	Total	9.720	7.332	8.364	10.003
College		7.328	6.897	5.211	5.696
Total	Menstruating	9.011	6.897	7.757	7.710
	Total	9.042	7.114	7.932	9.096
	Height				
High school	Non-menstruating	9.007	6.539	7.858	9.827
	Menstruating	8.836	6.880	8.681	9.423
	Total	9.720	7.332	8.364	10.003
College		7.328	6.897	5.211	5.696
Total	Menstruating	9.011	6.897	7.757	7.710
	Total	9.042	7.114	7.932	9.096

When pulse pressure is considered, the non-menstruating group of school girls leads with a correlation coefficient for weight of $+0.224 \pm 0.032$; for height, of $+0.168 \pm 0.033$ and for age, of $+0.144 \pm 0.033$.

The menstruating school girls and college girls show no significant correlation with either weight, height or age, and pulse pressure.

Unlike the blood pressure, the pulse rate in all cases in which the coefficient is significant, has a negative value. That is, as the factors with which it is correlated, increase, the pulse rate decreases. This would be expected.

In non-menstruating school girls, the greatest correlation exists between height and pulse rate, i.e., -0.186 ± 0.033. Age is next with $r = -0.170 \pm 0.033$ and weight has a coefficient of -0.163 ± 0.033.

When pulse rate is considered, however, it is not found, as for blood pressures, that the non-menstruating school girls show the greatest correlation. The menstruating school girls have a pulse rate-age correlation of -0.315 ± 0.029, and weight correlation of -0.125 ± 0.031. The coefficient for height is not significant ($r = -0.029 \pm 0.032$).

There is no significant correlation between pulse rate and either age, weight or height in college girls.

In drawing conclusions from this discussion, it is obvious that caution must be the keynote, since the fallacy of correlating groups in which a third factor vitiates the results obtained from correlating two, is so evident. There is always the possibility that the data placed in any one group may be still further separated by the consideration of other factors as yet not apparent.

Conclusions: 1. Blood pressure and pulse rate data for school girls and college girls, must be correlated separately. Those for school girls, must further be separated into that of a group of girls who have reached puberty and a group of girls who have not.

2. When systolic pressure is correlated with weight, height and age:

a. Non-menstruating school girls show the greatest correlation with the factors weight, height and age, in the order named.

b. Menstruating school girls show correlation, in this order, with weight and height but none with age. These coefficients are less than in *a*.

c. College girls show significant correlation only with weight.

3. When diastolic pressure is correlated with weight, height and age:

a. Non-menstruating school girls show the greatest correlation with the factors weight, height and age, in the order named.

The correlation, however, is not as great as that found in systolic pressure.

b. Menstruating school girls show correlation in the same order as in *a*, but the coefficients are less in each case.

c. Here too the college girls show correlation with weight only and this coefficient is less than that for systolic pressure.

4. When pulse pressure is correlated with weight, height and age:

a. The non-menstruating school girls show correlation with the factors in the order named. These coefficients are less than those for diastolic pressure.

b. The menstruating school girls and college girls show no significant correlation with any of the factors.

5. When pulse rate is correlated with height, age and weight:

a. The non-menstruating school girls show correlation with the factors in this order.

b. The menstruating school girls show a correlation with age and weight in this order.

c. The college girls show no significant correlation with any of the factors.

6. When considering data from girls who have reached the age of puberty, there seems to be in most cases, a better correlation, in all the series studied, in the school girl group than in the college group.

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THE PHYSIOLOGICAL ASSAY OF INSULIN BASED ON ITS EFFECTS ON THE HYPERGLYCEMIA FOLLOWING GLUCOSE INJECTIONS AND EPINEPHRIN

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The following paper really consists of two parts but these are published under one title since the main object of the investigations was the same, namely, to see whether some more precise method of physiological assay for insulin could be devised than that reported in a previous paper. The insulin in these experiments was given by the subcutaneous pathway.

I. THE EFFECT OF INSULIN ON THE POST-PRANDIAL GLUCOSE CURVE OF THE RABBIT. In view of the widespread use of the blood sugar curve following addition of glucose as a measure of the power of the body to utilize carbohydrate it was decided to investigate the effect of insulin on this curve, especially since it has been suggested that certain alterations observed in the curves are due to variations in the amount of internal secretion of the pancreas. The effect of insulin in lowering the hyperglycemia due to epinephrin, piqûre, asphyxia and ether (1) adds further interest to its possible effect on the hyperglycemia following injections of glucose.

Method: Details of the methods are the same as those given in a preceding paper (2). The only difference is that in this case insulin was injected subcutaneously in the lumbar region at various intervals before and after the glucose. The blood sugar was taken before giving insulin and again just before giving sugar.

The insulin used was derived from several sources and in the early stages of this investigation it was found impossible to preserve uniformity in the dose administered. As a result of this there was considerable variation in the blood sugar curves obtained in different observations when glucose was administered at the same interval after insulin. Later a larger supply of insulin was obtained and seven curves were made with this, using in each case 1 cc. of insulin per

kilogram of body weight. These rabbits were nos. 60 to 66 and are marked with an asterisk in table 1. The insulin was kept in the ice-chest during the course of these experiments.

Results: Although the results in which insulin preparations of varying potency were used are decidedly variable, they are given in tabular form (table 1) since several conclusions which have served as valuable guides to our further work can be drawn from them. The figures are to be compared with those of Ford and E. L. Scott (3) and of Eadie (2) in which it is shown that the administration of approximately 2.0 grams glucose per kilogram subcutaneously causes a rise in blood sugar in the first half-hour amounting on an average to 110 mgm., the extreme figures being 64 to 205 mgm.; and that the blood sugar returns to a value within the normal range in 3 to 5 hours or more. In the present table the results are grouped according to the length of the interval between giving insulin and glucose, both subcutaneously.

When glucose is given half an hour before insulin, the rise is of course of the normal extent, actually 182 mgm.; at this point insulin was given and in an hour the blood sugar was again normal. It continued falling until it reached 88 mgm., and then rose to normal.

Five observations were made after giving insulin and glucose together (nos. A, 32, 33, 47 and 60). Of these the first (rabbit 'A') is evidently abnormal and for some obscure reason the rabbit died during the experiment; it is therefore rejected. The other four show rises of 84, 17, 44 and 61 mgm. respectively, the rise of 44 mgm. being obtained with only 0.75 gram of glucose. These figures are lower than the average of those obtained without insulin, but because of the great variability noted above nothing very definite can be concluded. However, in every case the blood sugar has returned to normal within an hour and a half and in each case a marked hypoglycemia follows. Insulin thus causes the hyperglycemia to be less marked, particularly with regard to its duration when given simultaneously with sugar, but also often with regard to its intensity.

Three observations (40, 44 and 65) show the effect of glucose half an hour after insulin. To find the actual rise produced by the glucose in these cases one must subtract not the normal blood sugar value but the value obtained just before glucose was given. The rises were 71, 53 and 65 mgm., which are near the lower limit of normal rises and the normal level is reached again at the end of an hour and a half. In two of these cases hypoglycemia followed the hyperglycemia.

TABLE 1

NUMBER	DATE	DOSE GLUCOSE PER KGM.	INTERVAL BETWEEN GLUCOSE AND INSULIN	BLOOD SUGAR MGM. PER 100 CC.							
				Before insulin	Before glucose	After glucose					
						½ hour	1 hour	1½ hours	2 hours	3 hours	4 hours	5 hours
			hours									
46	28/7	2.0	− ½†	309	127	198	130	90	95	88	95	119
A	29/6	1.9	0	123		255	181	188	230	307	293	‡
33	4/7	2.0	0	131		215	125	99	97	93	77	72
32	11/7	1.8	0	142		159	101	119	94	86	95	
47	31/7	0.75	0	123		167	102	74	57	58	63	78
*60	25/10	2.0	0	114		175	136	105	79	59	56	65
40	17/7	1.9	½	117	92	163	109	82	72	92	97	115
44	17/8	2.0	½	137	101	154	116	115	116	109		125
*65	6/11	2.0	½	111	70	120	135	125	105	80	76	81
41	18/7	1.9	1	126	110	225	185		126	124	121	128
47	8/8	0.75	1	121	82	95	73	62	62	66	69	69
39	10/8	2.1	1	110	76	134	107	90	88	118	131	
40	10/8	1.9	1	154	114	199	195	191	181	179	186	
*62	30/10	2.0	1	109	102	139	135	121	100	68	96	94
36	9/8	2.0	1¼	128	67	89	85	82	71	60	99	107
37	9/8	2.0	1¼	94	28	67	67	79	75	66	61	
*61	28/10	2.0	1¼	105	56	109	58		54	52	55	
41	11/8	2.0	1½	117	90	136	121	107	98	101	93	95
43	21/7	1.9	1½	173	167	265	255	243	251	195	116	
42	11/8	1.9	1½	126	107	144	124	110	99	118	103	113
42	19/7	2.0	1½	95	89	208	201	163	142	110	123	120
*66	6/11	2.0	1½	110	46	96	107	86	50	70	56	54
43	14/8	2.0	2	167	89	158	173	172	188	183	162	140
45	14/8	2.0	2	102	86	136	133	117	114	114	114	117
*64	1/11	2.0	2	125	59	119	100	100	83	63	63	
35	26/6	2.1	2⅚	130	62	161	156	142	81	73		88
H1	15/8	2.0	3	120	90	93	142	135	149	148	117	117
H2	15/8	2.0	3	125	63	130	135	107	111	102	101	
*63	1/11	2.0	3	122	55	114	125	115	93	82	83	82
32	6/7	1.6	4	114	64	152	174	163	154	152	131	129
34	4/7	1.8	4¼	138	94	253	197	182	197	147	152	158
45	27/7	2.0	8	133	23	188	176	179				
35	29/6	2.1	3 days		135	305	249	194	156	142	130	141

† Half an hour before.
‡ Died.

Sugar was given in five observations 1 hour after insulin. In two cases (nos. 40 and 41) the insulin was very weak, as shown by the preliminary fall, and these two are omitted from consideration. In the other three rabbits the rises were 13, 58 and 37 mgm., the average (36 mgm.) being distinctly below the lower limit of the normal rise. In the case of rabbit 47, in an hour the blood sugar was below the level when the glucose was given and a marked hypoglycemia followed. In the case of rabbit 39 the blood sugar in an hour had returned only to about the level found before giving insulin and the subsequent hypoglycemia was less marked. Rabbit 62 shows only a slight fall before glucose was given, but the rise produced is quite small (37 mgm.) and it is followed by distinct hypoglycemia, the curve however falling rather slowly. This observation does not fit in with the series very well, and the slow fall before glucose was given is noticeable. This is probably partly due to slow absorption, but other results obtained by insulin injection in this particular rabbit point to its being due in part to an idiosyncrasy of the rabbit.

The insulin used when glucose was given one and a quarter hours after, was quite potent and its ability to suppress the hyperglycemia is clearly shown, the rises in these cases being only 22, 39 and 53 mgm., averaging 44 mgm. Since the normal rise is from 64 to 205 mgm., this and the preceding probably represent a real decrease in the rise and not an accidental occurrence.

Of the observations for one and one-half hour intervals, two were made with fairly potent insulin, viz., nos 41 and 66. Here the rises are 46 and 61 mgm. In one case the blood sugar has almost reached its normal level in an hour and there is a slight subsequent hyperglycemia. In the other it did not rise quite to its initial value throughout the experiment. Rabbit 42 on August 11th with slightly less potent insulin shows a very similar curve, with a rise of 37 mgm., lasting slightly over an hour and with subsequent hypoglycemia.

The three observations for the two-hour period were made with fairly potent insulin, judging from the preliminary fall, yet here the rises were 89, 50 and 60 mgm. In one case the normal level was reached in one hour, in one case in one and one-half hours and in the third, not again during the experiment although in five hours the blood sugar reached a value near the upper limit of the normal range. Decided hypoglycemia followed the increase due to sugar only in one case (no. 64).

At the three-hour interval the rises were 99, 52, 72 and 70 mgm. Noticeable here is the delayed rise shown most distinctly in no. H1,

and to a less degree in H2 and 63 so that the return to normal is slower than usual, occurring in one and one-half to three hours.

In four hours or more the effect of the insulin has worn off. The rise is of about the same height and the return to normal occurs after about the same interval as when glucose is administered without insulin.

The eight-hour curve is interesting on account of the extremely low blood sugar when the glucose was given. The rise here was 165 mgm. and the fall as far as it was followed does not give any indication of an effect from insulin. Evidently the extent of the rise from glucose does not depend on the level of the blood sugar when the glucose is given, and this curve shows that the blood sugar may be low as the result of insulin at a time when the insulin no longer has an effect on the injected sugar.

In general, in spite of their great variability, these results show that insulin affects the hyperglycemia due to injection of glucose, both in the extent of its rise and the duration, the effect on the duration being more marked when insulin and glucose are given together: that on the rise when the glucose is given one to one and one-half hours after insulin. This can be seen clearly in figure 1, in which are shown in curves the results of the observations in which equal doses of insulin were given (asterisked in table 1). The ordinates represent milligrams blood sugar and the abscissae two and a half-hour periods. Insulin was given at the beginning of each of the curves except the first one and 2 grams glucose per kilogram body weight at the times indicated. Curve 1 shows the effect of glucose without insulin and is drawn from data contained in a previous paper (2). From the extent of the initial fall in each of the other cases it is seen that tolerably uniform doses of insulin were injected.[1] After two hours the effect of insulin begins to weaken and it has almost disappeared in four hours. The effect at three hours however falls somewhat out of line.

Another series of similar curves is given in figure 2. Although the strength of insulin was not so uniform in these as in the preceding they are nevertheless important in that they show the same general results.

It is clear that the ability of the organism to assimilate from the blood an excess of injected glucose, following the subcutaneous injection of insulin, is most highly developed in a little over one hour after the injection. At this time, therefore, the strength of insulin might

[1] No. 62 is omitted since it is clear from the table that the insulin could not have been adequately absorbed in this animal; no. 32 is included since the initial insulin effect is comparable with the others.

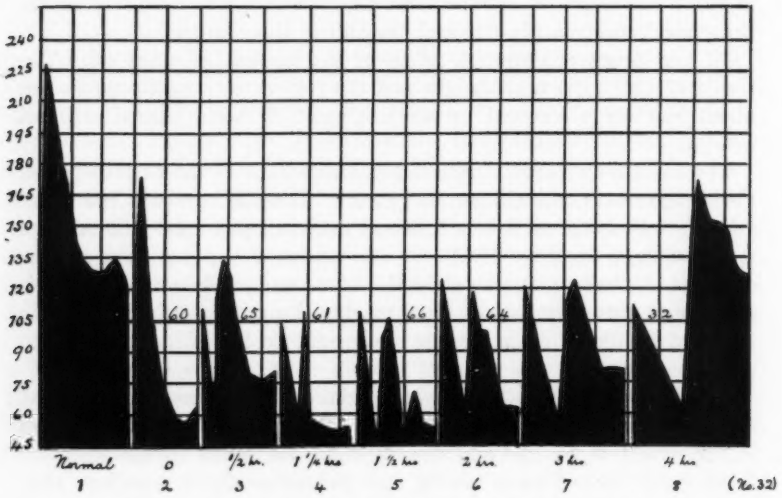

Fig. 1. Showing the effect of insulin on hyperglycemia produced by glucose: no. 1 is the blood sugar curve without insulin; in no. 2 the insulin and glucose were given together; in the remainder the glucose was given at the intervals named below, which on the curve correspond to the bottom of the first depression.

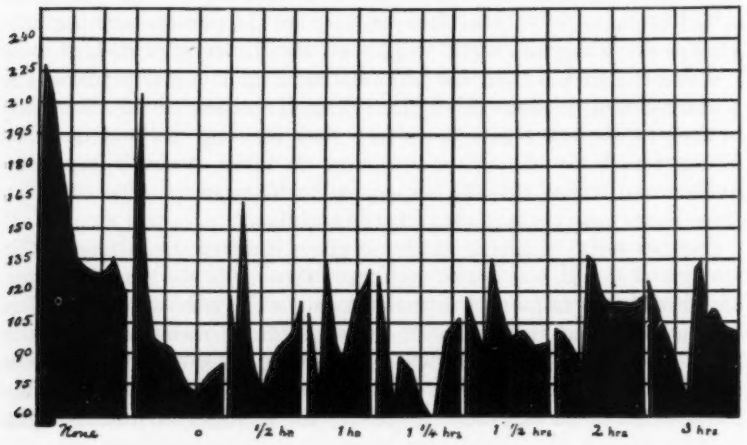

Fig. 2. Showing the effect of insulin on hyperglycemia produced by glucose: the significance of the numbers is the same as in figure 1.

be stated in terms of the maximal quantity of glucose which could be injected subcutaneously without causing any rise in the blood-sugar curve, or, for practical purposes, by measuring the extent and duration of the rise occurring after the injection of a fixed amount of glucose. For this purpose blood sugars would have to be taken at half-hour and one-hour intervals following the glucose injection. As one unit might then be considered the amount of insulin capable of preventing a rise of blood sugar to above the normal level of 0.115 per cent within one-half hour after the injection, subcutaneously, of 2 grams glucose per kilo body weight; the insulin being injected one hour prior to the glucose. Lesser doses of insulin than one unit would cause greater rises, the extent of which would probably indicate the dosage. This must, however, await further investigation.

SUMMARY

1. The hyperglycemia due to injections of glucose is affected by insulin both in degree and duration.

2. The effect on the duration is more marked when insulin and glucose are given together, that on the rise when the glucose is given one to one and one-half hours after.

3. A method for the physiological assay of insulin based on the results is suggested.

II. THE PHYSIOLOGICAL ASSAY OF INSULIN BASED ON ITS EFFECTS ON EPINEPHRIN HYPERGLYCEMIA. Since insulin markedly reduces the hyperglycemia produced by subcutaneous injections of epinephrin (4), it was thought that this might prove the basis of a method for the pharmacological assay of insulin. In the experiments of the previous section this assay was attempted by finding how much insulin would be required to prevent the rise in blood sugar following the injection of 2 grams per kilo of glucose injected subcutaneously—i.e., of exogenous origin—and in the present experiments the same principle was followed with the difference that the glucose was supplied from endogenous sources by causing increased glygogenolysis through the action of epinephrin. A similar method of assay was used by Zuelzer (4) in testing the antidiabetic potency of the alcoholic extracts of pancreas which he investigated. There can be no doubt that Zuelzer succeeded in demonstrating that such extracts may contain substances capable of depressing the degree of hyperglycemia due to epinephrin but he failed to isolate from these extracts other substances having a deleterious influence so that the investigations were not further continued.

Method: Rabbits weighing as closely as possible 2 kgm. were taken from the pens twenty-four hours before the experiment was to begin and placed in cages away from solid food, but with a supply of water. Varying doses of insulin were then injected subcutaneously in the lumbar region, a sample of blood having been taken just before. Since in the preceding section it was shown that insulin given subcutaneously develops its maximum effect in about an hour and a quarter, epinephrin was given at this interval after insulin. The preparation used was taken from hypodermic ampoules (Parke Davis Co.) and 1 cc. given in each case. Further samples of blood were taken just before the epinephrin, and at intervals of one-half, one and two hours after. The blood sugar was measured by the Shaffer-Hartmann method.

Two different lots of insulin were used. The first one (lot 143) was standardized by finding the minimal dose necessary to produce in rabbits a lowering of blood sugar to about 0.045 per cent and convulsions in two to four hours as described previously (5). The second (lot 6) was standardized by comparing the results obtained by it with those of the first lot. Both lots were kept in the ice-chest and portions removed when required under aseptic precautions.

Results: A summary of the results is given in table 2. The dose of insulin per kilogram was calculated by the effect on blood sugar and the incidence of convulsions (5), thus, in "A" 0.2 cc. insulin per kilo contained one unit and in "B", 0.75 cc. The figures in the column headed "fall" were obtained by subtracting the blood sugar values before epinephrin from those before insulin, and give the fall of blood sugar produced by insulin in an hour and a quarter; those in the various columns headed "rise," by subtracting the blood sugar value before epinephrin from the values one-half, one and two hours after it respectively, and show the rises produced by exactly the same doses of epinephrin in those periods.

The relation between the dose and the fall of blood sugar produced by it is dealt with elsewhere (5). In each case but one the epinephrin produces a rise in the blood sugar and the rise is roughly proportional to the dose of insulin. In the one case (rabbit 82) there is an actual fall, the cause for which we are unable to explain.

On plotting the dose of insulin against the rise produced by epinephrin in each case no simple relation holds between them as will be seen in figure 3 in which the values for the two-hour period are plotted along with a curve which will be described immediately. On the other hand, when the logarithms of the doses are plotted against the loga-

rithms of the rises occurring in the one and two-hour periods the majority of them fall along a straight line. It was therefore attempted to find an empirical formula by which the dose might be calculated from the rise and which might be useful for purposes of assay.

Since the figures for the two-hour period were slightly closer than those for the other periods these figures were chosen for the construction of this formula. To make the figures easier to work with log $20\ d$

TABLE 2

RABBIT NUMBER	DATE	INSULIN PER KGM.	DOSE INSULIN PER KGM.	BLOOD SUGAR MGM. PER 100 CC.								
				Before insulin	Before epinephrin	Fall	After epinephrin					
							½ hour	Rise	1 hour	Rise	2 hours	Rise
A. Insulin, lot 143, 1 dose in 0.40 cc.												
		cc.										
70	11/10	0.2	0.5	117	84	33	145	61	221	137	293	209
71	11/10	0.4	1.0	120	59	61	86	27	94	35	112	53
72	16/10	0.8	2.0	127	62	65	76	14	82	20	82	20
73	16/10	0.8	2.0	119	55	64	66	11	69	14	73	18
B. Insulin, lot 6, 1 dose in 0.74 cc.												
74	18/10	0.93	1.2	104	5	99	20	15	41	36	38	33
75	18/10	0.51	0.7	112	17	95	30	13	58	41	91	74
76	19/10	0.56	0.8	107	57	50	92	35	149	92	234	177
77	19/10	0.23	0.3	92	59	33	114	55	210	151	287	228
79	23/10	0.40	0.5		92		148	56	186	94	279	187
80	7/11	1.0	1.4	100	66	34	111	45	134	68	149	83
81	7/11	0.93	1.2	106	57	49	77	20			79	22
82	8/11	1.17	1.6	111	69	42	48	Fall			53	Fall
83	8/11	1.50	2.0	121	28	93					45	17
84	10/11	1.50	2.0	125	38	87	42	4	43	5	54	16
85	10/11	1.80	1.8	122	46	76	76	30	104	58	159	113

was plotted against log r. A straight line passing through the middle of these points was then found to be represented by the equation

$$\frac{\text{Log } 20\ d}{2.38} + \frac{\text{Log } r}{3.84} = 1.$$

where d = number of doses per kilogram and r = rise of blood sugar in milligram per 100 cc. in two hours. By multiplying through to get

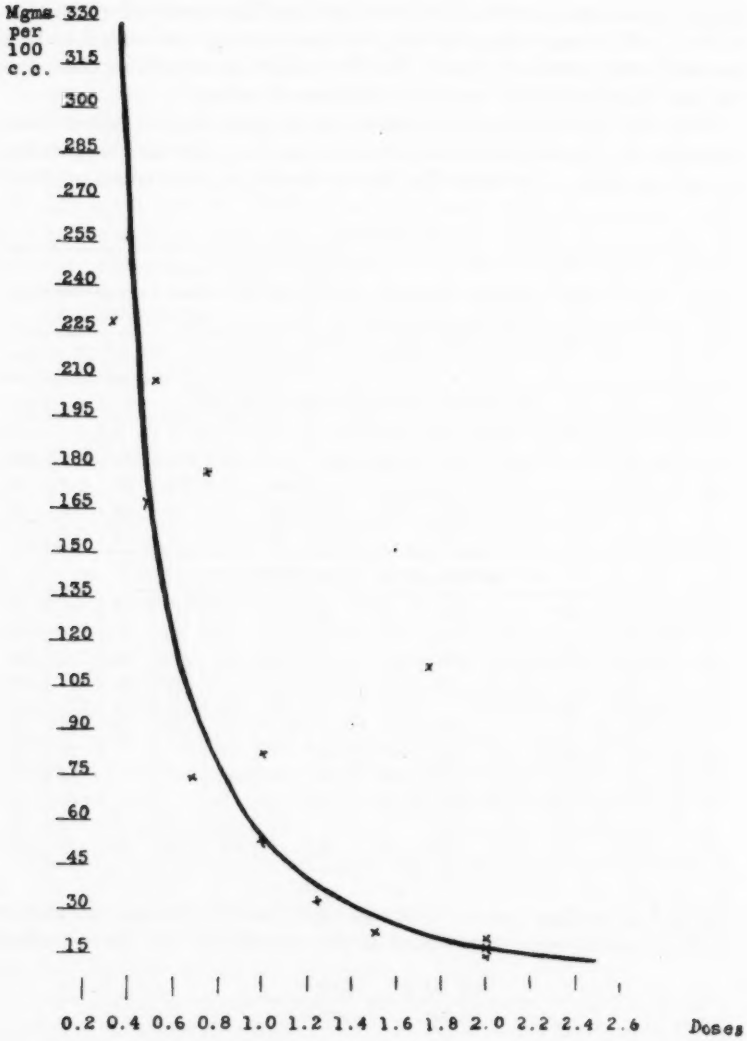

Fig. 3. Showing the relation between the dose of insulin and the rise produced by the standard dose of epinephrin. The ordinates give the rise in blood sugar following epinephrin and the abscissae the doses of insulin. The crosses represent observations; the curve is drawn from the equation $d\ (r)^{0.62} = 10^{1.08}$.

rid of fractions and changing it from the logarithmic to the exponential form we get an equation of the type $d \cdot (r)^a = K$ and in this case $a = 0.62$ and $K = 10^{1.08}$. The values for K in each case are given in table 3. The curve corresponding to this equation is also plotted in figure 3, from which it will be seen that only one point is widely divergent; one other figure (rabbit 82, table 3) is not plotted, for here it will be recalled there was an actual fall instead of a rise after the epinephrin. To show how far this is useful in calculating the dose of insulin used, table 4 was constructed in which the third column gives the actual dose of insulin used and the fourth the dose calculated by the formula.[2] The

TABLE 3

RABBIT NUMBER	K	d (ACTUAL)	d (CALCULATED ON BASIS OF $K = 10^{1.08}$)
70	$10^{1.1}$	0.5	0.4
71	$10^{1.1}$	1.0	1.0
72	$10^{1.1}$	2.0	1.9
73	$10^{1.1}$	2.0	2.0
74	$10^{1.0}$	1.2	1.1
75	$10^{1.0}$	0.7	0.8
76	$10^{1.3}$	0.8	0.5
77	$10^{0.9}$	0.3	0.4
78	$10^{1.1}$	0.5	0.5
80	$10^{1.3}$	1.4	0.9
81	$10^{0.9}$	1.2	1.8
83	$10^{1.1}$	2.0	2.1
84	$10^{1.0}$	2.0	2.2
85	$10^{1.4}$	1.5	0.6

results are fairly close in most instances; a few however are quite widely divergent. This is obviously due to a biological variation and depends on some as yet unknown factor, for in spite of careful standardization of rabbits it was found impossible to avoid this variation. It may be possible to reduce this divergence by giving the insulin intravenously.

This method therefore cannot be counted on to give reliable results on any individual rabbit and in using it a number of rabbits must be employed if a trustworthy figure is to be obtained. In spite of this the figures show that a relation of the type $d \cdot (r)^a = K$ holds between the dose of insulin and the rise following epinephrin.

[2] In calculating the dose either this formula or the curve may be used. In the latter case the point on the curve corresponding to the rise in two hours is found, and the dose is then read off along the other axis.

The figures for the one-hour period on investigation were found to obey, but less closely, the same law; the constants however are different. Those for the half-hour period on the other hand are very widely divergent and for such purposes as these are quite useless.

SUMMARY

1. Figures are given showing the extent of the hyperglycemia produced by epinephrin administered an hour and a quarter after varying doses of insulin.

2. It is shown that the rise produced in two hours by epinephrin (r) is related in general to the dose of insulin (d) by the empirical formula $d \cdot (r)^a = K$, in which a and K are constants having the values 0.62 and $10^{1.08}$ respectively.

3. Owing to the unavoidable biological variations of the animals this formula cannot be used for assaying insulin, unless a series of animals is used so that there is no practical advantage over the method previously proposed (5).

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THE EFFECT OF A RESTRICTED DIET

V. On Mortality, Cannibalism and the Sex Ratio

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In our former papers on the effect of a diet restricted practically to plant sources we presented our results on the following topics: I, on growth (1): II, on the age of puberty, the menopause, and on sterility (2); III, on the number of litters and young born (3); and IV, on the age of greatest productivity (4). In this paper we will give our results on the effect of the restricted diet on the relative mortality, cannibalism and the sex ratio of the young.

The method of procedure in our experiment was given in detail in our first paper, but in order to have it clearly in mind it will be briefly summarized.

To facilitate handling the data we divided our matings into five groups, based on the origin of the stock from which the individuals of the pair were taken. The control rats, or omnivorous feeders, have been designated group I. Those matings in which both male and female were selected from the omnivorous stock and had been on an omnivorous diet until the time of mating but thereafter were on a restricted diet were placed in group II. When one rat of a pair was taken from the control stock and its mate from the offspring of the restricted feeders, such mating constituted group III. All matings in which both sexes were selected from the young of restricted feeders and had been on a restricted diet since birth formed group IV. Group V was composed of those restricted feeders which toward the end of the experiment were given an omnivorous diet.

All groups of our rats were subjected to the same external environment. They were all fed daily the same kind of plant food consisting largely of table scraps, green vegetables, nuts, cracked corn, etc. Two or three times per week our controls had, in addition, food from animal sources, such as scraps of meat and eggs and some milk, etc. A résumé of the literature shows that a mixed diet such as the average man

usually eats contains not only the necessary calories supplied by the proteins, carbohydrates and fats consumed, but also apparently furnishes all the accessory factors (vitamins) for his needs. The table scraps given to the restricted feeders were sorted as carefully as possible and bits of meat, eggs, etc., removed. However, some foods from animal sources could not be wholly eliminated, such as seasoning of butter, animal fats, milk, etc. With these exceptions the diet of our restricted feeders was derived from plant sources. It corresponded fairly closely with the so-called vegetarian diet of human beings.

The only difference in the treatment of our controls and restricted feeders therefore was the presence of a greater amount of food from animal sources in the diet of our controls over that of the restricted feeders. Any modifications in the results of the omnivorous and restricted feeders must therefore be attributed to the difference in diet, since all other environmental conditions were the same and all the rats were from the same stock.

In breeding experiments with swine Carmichael and Rice (5) have shown that older sows farrowed a higher per cent of dead and immature pigs than younger sows. Of the pigs born dead 56 per cent were males. This seems to indicate that the vitality of the young decreases with the increase in the age of the mother and that the vitality of the males is less than that of the females. They also found that in the 5657 pigs observed, 51.9 per cent were males and 48.1 per cent were females. When the data regarding sex ratio were arranged in serial order they found in the 261 litters observed that the percentage of males was greatest in the first litters and least in the last, being 59.4 and 51 per cent respectively.

The results of different investigators concerning the sex ratio in the albino rat show some variations. Cuénot (6) found in 255 young albino rats a ratio of 105.6 males to 100 females. King and Stotsenburg (7) gave a ratio of 107.5 males to 100 females for 1089 litters born largely from young stock albino mothers that had not reached the height of their reproductive activity. King (8) states that since the sex ratio of young albino rats apparently depends to a certain extent on the age of the mother, the above figures do not represent the normal average ratio. In a later paper (9) she has given the ratio of males to 100 females for the first four litters as follows: 115.9, 102.2, 95.6 and 105.9 respectively. The average ratio for the first four litters was 104.6 males to 100 females, or approximately 105 males to 100 females. This she has taken as the normal for comparison with the first four

litters of a series of inbred rats. Since the sex ratio varies with the age of the mother, the norm of King would be somewhat modified by the data from later litters and the relative number of males would be less.

In regard to the mortality in young stock rats, King (10) states that in the males 1.1 per cent died before six months, 14.1 per cent before nine months, 36.2 per cent before twelve months, and 71.9 per cent before fifteen months of age. In regard to the females, 4.5 per cent died before six months, 12.4 per cent before nine months, 29.8 per cent before twelve months, and 62.4 per cent before fifteen months. No data are given regarding the number which died before the age of puberty.

That the health of individuals was greatly affected by enforced dietary conditions in Germany during the late war has been shown by Starling (11). He states that there was a widespread increase of tuberculosis, causing deaths from this disease to increase from two and one-half to six times.

The effect of diet on mammary secretion has been studied by Hartwell (12). He states that the amount and quality of mammary secretion is affected by diet. An increase in the mortality of the young due to inadequate food may therefore result. Abderhalden (13) states that when rats were fed on polished rice various diseases developed and death resulted in from 100 to 200 days. Of the 174 females used only 14 gave birth to young, all of which died within 103 days.

Many other investigators have shown that inadequate diets are the cause of many diseases and an increase in mortality.

On a diet in which corn furnished the source of protein and ash, Hogan (14) found that reproduction occurred in rats but that they consistently ate their young for five litters, at which time their diet was changed. It is commonly known among investigators using rats for experimentation that the young are often eaten by the parents. King (9) states that in order to obtain accurate sex data of all rats born it is necessary to determine the sex as soon after birth as possible, for the young that are still-born, or those that die soon after birth, are usually eaten by the mother within a few hours. We have had many instances of cannibalism, not only in young rats but also in older and mature individuals. Often when one of a pair died it would be found at the next feeding time to have been partly eaten by its mate.

The results of many other investigators might be given which illustrate normal results and how these results may be affected in various

ways by changes in the diet. They all agree, however, that modification of the diet of an animal may change the percentage of mortality, cannibalism and possibly the sex ratio.

MORTALITY OF YOUNG. During the course of the experiment the omnivorous feeders produced 1239 young. Of this number 457, or 36.4 per cent, died before reaching sexual maturity. This percentage of mortality is slightly greater than it would have been if the animals had been fed throughout the experiment on the rich omnivorous food used during the major portion of the time. During the last six or eight months of the experiment the amount of animal protein given was somewhat reduced, with a resulting increase of mortality from 36.4 to 49.0 per cent. The figures above include this period of semi-restricted feeding. When this period is eliminated the per cent of mortality of the young is reduced to 35. Since these last few months constituted only a small part of the whole time and the diet was only slightly restricted, the data for the whole period of over eight years will be used to represent the normal omnivorous feeders. The condensed results showing the mortality of the young are tabulated in table 1.

If we compare the average results of all matings placed on a restricted diet (which include groups II, III, IV and V) with the results obtained from the omnivorous group we note some marked differences. The number of pairs (130) was almost one and one-half times as many as those of the omnivorous group, but the total number of young born (525) is only 42.4 per cent of the number of the controls, or a ratio in productivity of approximately 2.65 to 1. Of this 525 young, 347, or 66.1 per cent, died before reaching sexual maturity. The ratio of mortality of the normal to the restricted feeders was thus 1:1.8. That is, the mortality of the young was increased almost twofold by the restricted diet.

When the different groups of the restricted feeders are considered, we find the results somewhat variable. By consulting table 1 and comparing groups II, III and IV in regard to the mortality of the young we find there was a progressive decrease in the order given. The relatively high mortality (78.6 per cent) in group II indicates that the sudden change from an omnivorous diet to a restricted one is more fatal to their young than to the young of parents that had been on a restricted diet for some time. This may have been due to a weeding out of the weaklings in group IV, leaving only the strong and more vigorous to breed. A certain strain may thus have been propagated

TABLE 1

Showing the comparative number of young born in the different groups of matings, the per cent of mortality of the young, the per cent of dead that were eaten, the ratio of the number living to the number eaten, and the sex ratio

	NUMBER OF PAIRS	PER CENT BREEDING PAIRS	NUMBER OF YOUNG		PER CENT OF MORTALITY OF YOUNG	NUMBER EATEN	PER CENT OF DEAD EATEN	NUMBER OF LIVING TO 100 EATEN	SEX RATIO			
			Born	Died					Number of litters used	Number males	Number females	Number males to 100 females
Omnivorous, Group I	90	89.0	1239	451	36.4	332	73.6	292	277	572	529	108.0
All matings on restricted diet groups II, III, IV, V	130	47.5	525	347	66.1	265	76.3	165	138	190	195	95.5
Omnivorous stock on restricted diet group II	67	57.0	229	179	78.6	140	78.2	131	52	73	87	84.0
1 Omnivorous, 1 restricted on restricted diet group III	30	37.0	96	65	67.7	55	84.6	165	24	40	25	116.0
Restricted stock on restricted diet, group IV	19	50.0	91	52	57.2	43	82.7	209	23	24	28	86.0
Restricted stock on omnivorous diet, group V	14	79.0	109	58	53.2	41	70.7	210	34	53	59	90.0
First and second generation restricted stock on restricted diet	26	25.0	99	64	64.7	44	68.7	165	20	38	39	97.0

which was more able to endure the adverse conditions imposed by the restricted diet. The difference might also have been due to a possible readjustment to the less favorable diet which the animals may have been able to make after some time had elapsed. This assumption is strengthened when the mortality of the successive litters of group II is compared. By consulting table 2 we find that the mortality of the first, second and third litters of group II was 82.5, 76 and 37.5 per cent respectively. The great reduction in mortality occurred between the second and third litters. If a readjustment occurred it must have been most effective after the birth of the second litter. Since the average age at mating of group II (which was the time the animals were placed on the restricted diet) was 157 days and the average age at birth of the second litter was 272 days, the supposed readjustment required about four months, or about one-eighth of the average life span of the rat.

In group V there was a marked increase in the number of young born per pair, and in the vitality of the young as indicated by the per cent of mortality. This group, however, still had a much higher per cent of mortality than the controls. This noticeable improvement was apparently due to an increase of food from animal sources given this group during the last few months of the experiment.

When the mortality of all the young of those matings made from the first and second generations of restricted feeders was considered we found that it compared closely with the average of all restricted feeders. By consulting table 1 we see that the mortality of the 99 young born to those matings was 64.7 per cent. This is greater than the mortality of group IV and shows in this respect the progressive effect of the restricted diet. Our data show that the percentage of mortality of the young cast by the first litters of the first generation of group II was greater than that of the young thrown by the second and third litters. This again points to the assumption that there was either a weeding out of the weak or an adaptation to the adverse conditions which enabled the mothers to cast more resistant young. Possibly both these factors were instrumental in reducing the mortality of the young of later litters.

Only four matings were possible which included offspring of the second generation. Of these four pairs, 75 per cent, failed to reproduce and the young of the fourth pair had a mortality of 100 per cent. These results show that rats fed on a diet practically restricted to plant sources failed to reproduce beyond the third generation and the line of descent became extinct.

In order to determine whether some of the litters showed a greater mortality of young than other litters the data were arranged as in table 2. Examination of this table shows that the per cent of mortality of the different litters of the omnivorous group ran fairly constant until the sixth litter when the per cent of the number which died increased. The greatest mortality was in the seventh litter. In the eighth litter the mortality was less than in the seventh, and in the ninth had decreased to a little less than that of the first five litters. The number of individuals in each of the last four litters, however, is too small for the results to be wholly trustworthy, as the increase and decline in mortality of the young for the sixth, seventh and eighth litters may have been due to individual variation of the few females involved. But somewhat similar results were obtained in the mortality of the young of all matings (groups II, III, IV, and V) on restricted diet and in groups III and IV. This suggests that the vitality of the young varies with the age of the parents.

CANNIBALISM. It is a common custom with the wild rats to eat their dead. This habit is also very prevalent in the albino rat. Not only is it common for them to eat the dead, but cases have been observed where the parents killed and ate the new-born. This occurs within a few hours after the young are born. It is therefore impossible to get a correct estimate of the number eaten, unless the rats are under continual observation. Our results are from observations made at intervals during each day and to this extent are complete. Though the young are more subject to this cannibalistic habit, we have noted many cases where dead rats of all ages have been partly or completely devoured by the other occupants of the cage.

In the omnivorous group (table 1) 332, or a little over two-thirds of those which died, were eaten. This represents 26.8 per cent of the total young born and 73.6 per cent of those which died. These 332 dead were eaten by 968 individuals which included the 180 parents and the 788 living young. The ratio of dead eaten to the living individuals was 1 to 2.92. That is, it required almost three living rats to dispose of one dead one. This habit seems to be due to a craving for animal protein. If this protein is present in sufficient amount in the food the number of dead eaten is reduced. If the supply of animal protein in the food decreases the number of dead eaten increases. However, some individuals persisted in eating their young regardless of the character of the diet. The increase in cannibalism is very noticeable when we compare the number eaten by the normal rats

TABLE 2

Showing the average per cent of mortality of the young and the per cent of the young eaten in the different litters born in the different groups

MATINGS		LITTERS								
		1	2	3	4	5	6	7	8	9
Omnivorous (group I)	Number of pairs	90.0	65.0	49.0	31.0	17.0	10.0	8.0	5.0	2
	Number of young born	352.0	359.0	225.0	151.0	66.0	36.0	27.0	17.0	6
	Per cent mortality	35.0	36.2	36.8	34.5	34.8	47.2	63.0	41.0	33
	Per cent eaten of total born	23.8	29.0	30.6	19.9	27.2	36.1	33.3	29.4	33
	Per cent of dead eaten	66.0	81.0	81.5	58.0	78.0	59.0	70.5	71.5	100
All matings on restricted diet (groups II, III, IV and V)	Number of pairs	62.0	39.0	16.0	2.0					
	Number of young born	299.0	162.0	54.0	11.0					
	Per cent mortality	68.6	74.0	52.0	18.2					
	Per cent eaten of total born	54.0	53.0	29.5	18.2					
	Per cent of dead eaten	72.0	71.5	57.0	100.0					
Omnivorous on restricted diet (group II)	Number of pairs	20.0	9.0	3.0						
	Number of young born	97.0	55.0	16.0						
	Per cent mortality	82.5	76.0	37.5						
	Per cent eaten of total born	71.0	60.0	6.25						
	Per cent of dead eaten	87.0	78.6	17.0						
1 omnivorous, 1 restricted on restricted diet (group III)	Number of pairs	8.0	8.0	4.0						
	Number of young born	32.0	40.0	15.0						
	Per cent mortality	51.0	65.0	40.0						
	Per cent eaten of total born	44.0	65.0	26.6						
	Per cent of dead eaten	87.5	100.0	67.0						

Restricted on restricted diet (group IV)	Number of pairs	10.0	6.0	5.0						
	Number of young born	43.0	22.0	15.0						
	Per cent of mortality	53.0	68.0	60.0						
	Per cent eaten of total born	44.0	59.0	53.0						
	Per cent of dead eaten	82.5	86.7	89.0						
Restricted on omnivorous diet (group V)	Number of pairs	10.0	12.0	4.0	2.0					
	Number of young born	45.0	45.0	8.0	11.0					
	Per cent of mortality	67.0	49.0	87.5	18.2					
	Per cent eaten of total born	42.0	31.0	37.5	18.2					
	Per cent of dead eaten	63.4	63.7	43.0	100.0					

with those eaten by the combined restricted groups. Here the per cent of dead eaten increased from 73.6 in the normal to 76.3 in the average of the restricted groups. In other words, of the 347 which died in the restricted group, 265 were eaten by the 438 remaining living rats. These figures form a ratio of those eaten to those living of 1 to 1.65. Cannibalism in the average of all restricted groups had thus increased almost twice that of the normal when the ratios of the eaten to the consumers were compared. If cannibalism had existed to the same degree in our controls practically all the dead would have been eaten.

When the different groups of the restricted feeders are considered we find the results somewhat variable. In group II the per cent of dead eaten was 78.2, and the ratios of those eaten to the consumers was 100 to 131. In this case the ratio approached more closely to a 1:1 relationship than in any of the groups. We attribute this to the craving for animal food of which this group was suddenly deprived at mating. Group III had the greatest per cent of dead eaten of any of the restricted groups. The craving for animal food as indicated by the ratio of those eaten to the consumers, however, was not so great as in group II. This ratio falls about midway between that of group II and of group IV. In group IV, though the per cent of dead eaten is high the ratio of the eaten to the living of 1:2 shows a decrease in the cannibalistic habit. This seems to indicate that some adjustment had taken place in this group which resulted in a reduction of the craving for animal food. In group V we attribute the reduction in the per cent of dead eaten to the increase of food from animal sources given this group during the last few months of the experiment. Cannibalism, as shown by the ratio of eaten to living, however, was still above normal.

In order to determine whether cannibalism is more prevalent at one age than another the data for the average successive litters have been arranged in table 2. By consulting this table it is seen that in the control rats (group I) the per cent of dead eaten varied with successive litters, but in general increased with age. The last litter showed the highest per cent. This also occurred in group IV. But in group II the first litter showed the highest per cent of dead eaten, and the last litter the lowest per cent. This seems to substantiate the assumption made above, that a readjustment to adverse conditions had taken place, which resulted in a decreased craving for animal food. The results of group V seem to indicate a progressive reduction in the craving for animal food up to the third litter. At this time the richer diet was given which apparently did not satisfy the animals but seemed to act as a stimulus to increased cannibalism.

SEX RATIO. In order to determine the correct sex ratio of all young rats born it is necessary to decide the sexes at birth. This is because of the prevalent habit the parents have, especially the mothers, of eating their young within an hour or two after birth. Many cases of still-birth and of young which died soon after birth would thus be unrecorded if the examination and determination were delayed. Our data were compiled from observations made at various times during the day. Our results, therefore, may possibly deviate somewhat from those which might be obtained from continuous observation. They are, however, comparable in respect to the different groups considered. The sexes were not determined in all our litters.

By consulting table 1 it is seen that the average sex ratio of all litters of all matings of our controls, or omnivorous feeders, was 108 males to 100 females. This is a somewhat higher percentage of males than that given by other investigators. If we compare the different groups of the restricted feeders with our controls we find that with the exception of group III the percentage of males is very much reduced. The number of males range between 84 and 97 for each 100 females. We are unable to give a reason for the high proportion of males in group III. These data show that in general the restricted diet had a marked influence in reducing the number of males in proportion to the females.

In order to determine if the age of the parents had any influence on the sex ratio our data for each of the average litters have been arranged in table 3. This table shows that the ratio of males to females in the successive litters of our controls was quite variable and showed wide extremes. This is probably due to the lack of a sufficient number of determinations to give accurate averages. If the first three litters are compared with the last three we can say that young females tended to throw a greater number of males than did older females. These results also show that the second litter had a slightly greater proportion of males than the first. The successive litters after the second showed, with the exception of the sixth, a general reduction in the percentage of males. When the average litters of the combined groups of restricted feeders were considered, we found somewhat similar results in that the second litter had a greater proportion of males than the first, but the first had the lowest proportion of any litters cast. No very definite conclusions can be drawn from the data concerning the different litters. The results, however, seem to indicate that the percentage of males is highest in the second litters of both controls and restricted feeders, and less in later litters. Also that the restricted diet caused

TABLE 3

Showing the average sex ratio of the different litters used in the controls and all groups of the restricted feeders

		LITTER								
		1	2	3	4	5	6	7	8	9
Controls—omnivorous feeders, group I	Number of litters	53.0	46	39.0	28.0	15	9	5	5	2
	Number of young	268.0	238	175.0	135.0	57	29	19	17	6
	Number of males	142.0	127	92.0	61.0	30	18	9	9	3
	Number of females	126.0	111	83.0	74.0	27	11	10	8	3
	Number of males to 100 females	112.0	114	110.5	82.5	111	162	90	112	100
All restricted feeders. Groups II, III, IV, V	Number of litters	48.0	16	6.0	1.0					
	Number of young	213.0	76	26.0	6.0					
	Number of males	104.0	43	13.0	3.0					
	Number of females	109.0	33	13.0	3.0					
	Number of males to 100 females	95.5	130	100.0	100.0					

a notable reduction of males in the first litter and a marked increase in the second as compared to the percentage of males in the first and second litters of our controls.

CONCLUSIONS

From the above results the following general summary may be deduced in regard to the effect of a diet restricted practically to plant sources, in regard to mortality, cannibalism and sex ratio:

1. In our control rats the mortality of the young was greatest in the older litters.

2. The restricted diet caused an increase in the mortality of the young.

3. The mortality of the young was greatest in the first litters, less in the second, and least in the third litters cast by parents from omnivorous stock placed on the restricted diet (group II). This indicates a possible adjustment to the inadequate diet, which required about one-eighth of the averate life-span of the rat and appeared to result in more resistant or stronger young.

4. The mortality of the young of the second generation matings of the restricted feeders was 100 per cent.

5. The line of descent of the restricted feeders became extinct by the third generation.

6. In our control rats there was in general an increase of cannibalism as the parents grew older, being greatest in the last litters. This was also true in one group of restricted feeders whose parents were from restricted stock (group IV).

7. Cannibalism was influenced by the diet. In general, it was more prevalent in the animals deprived of food from animal sources.

8. A sudden change from the omnivorous diet to the restricted diet (group II) caused a marked increase in cannibalism in the first litters. The last litters had the least per cent of young eaten. This again suggests that some adjustment had been made by the parents, which appeared to reduce this craving for food from animal sources.

9. The average sex ratio in our control rats was 108 males to 100 females. The second litter had a greater proportion of males than the first litter. In general, the later litters had a reduction in the proportion of males.

10. The general effect of the restricted diet was to greatly reduce the proportion of males. With the exception of one group, the males ranged between 84 and 97 to 100 females.

11. The results of the whole series of papers show that, owing often to a long delay in the effect, feeding experiments should be extended over a considerable portion of the life-span of the animal, to be conclusive.

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VASODILATOR MECHANISMS

IV. The Intra-Arterial Injection of Histamine

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When nicotine is injected into the arterial blood stream in such a manner that it acts directly on the peripheral blood vessels and only secondarily and after an appreciable delay reaches the vasoconstrictor center and the sympathetic ganglia, its primary effect is vasodilatation (1). This was demonstrated by injecting the toxin centrally in the right internal iliac artery and thus forcing it past the bifurcation of the aorta into the blood stream going to the left leg which was incased in a plethysmograph. After several doses of nicotine have been administered in this manner the blood vessels will no longer respond to that toxin.

It was suggested that nicotine might possibly act on the endothelium of the capillaries in the same manner as on sympathetic ganglia, first causing stimulation and later paralysis. Since histamine is regarded as a poison for endothelium and causes, according to Dale (2), a relaxation of the capillary wall, it seemed desirable to compare its action with that of nicotine.

The *technique* employed in this investigation is the same as that described in previous papers of this series (1), (3). The substances to be tested were injected toward the aorta through the central end of the right iliac artery, which had been ligated and drawn through an opening in the flank. The injected substance was forced past the bifurcation of the aorta and carried with the blood stream to the left leg and left side of the pelvis. The left leg was incased in a plethysmograph with a complete rubber stocking; and the space between the stocking and plethysmograph was filled with warm water. The advantages of intra-arterial injection are obvious. It enables one to study the response of a restricted vascular area while all other parts of the circulatory system, including the vasomotor center, remain unaffected.

There were two groups of experiments. In the first series of dogs the left leg was denervated at the beginning of the experiment. The obturator and femoral nerves were cut inside the pelvis, the left abdominal sympathetic trunk removed, and the sciatic cut outside the pelvis. In the second series the nerves were cut under aseptic precautions and time allowed for degeneration before the tests were made. In these dogs the sympathetic filaments accompanying the iliac vessels were divided in lieu of removal of the sympathetic trunk.

SERIES I. *Nerves cut but not degenerated.* When injected centrally through the right iliac artery $\frac{1}{10}$ mgm. of histamine is sufficient to cause a nearly maximal vasodilatation in the left leg. With doses of less than 1 mgm. there regularly occurs along with the swelling of the leg a very considerable increase of the pulse waves in the plethysmographic tracings. This increase is often as marked as that caused by acetylcholin. In figure 1 the height of the plethysmographic pulse is fully twice as great at the height of the reaction as before the injection. If, as postulated by Dale, acetylcholin acts on the arteries and histamine on the capillaries, the vasodilatation caused by acetylcholin should be accompanied by a much greater increase in the pulse wave than is a dilatation of the same degree produced by histamine. But this is not the case. If the large doses of histamine which cause arterial constriction are avoided there is very little difference in the effect of the two on the pulse. Even when doses as great as 2 mgm. are given, an increase in the pulse waves is usually seen during the first part of the reaction, but this is often followed by an arterial constriction and a decrease in the plethysmographic pulse. Dale and Richards (4) attributed the dilator action of histamine to its action on the capillary wall, yet they add that their evidence does not warrant a sharp distinction in this respect between capillaries and arterioles. We believe that the marked increase in the pulse waves seen in the plethysmographic tracings after histamine injection is evidence that at least the smaller arterioles are involved in the reaction. When capillaries are observed under the microscope in the living animal they do not show pulsations. Even in cases of aortic regurgitation, which showed the so-called capillary pulsation, Boas (5) could not demonstrate any pulsation in the capillaries when these were examined with the microscope. He believes that the flushing which is observed clinically in such patients and which is synchronous with the pulse has its origin in the terminal arterioles and not in the capillaries.

Rich (6) studied the effect of histamine on the blood vessels in the omentum, fixed in situ during the height of the reaction and when examined microscopically he found the arterioles as well as the capillaries dilated. Carrier (7) observed the capillaries in the human skin at the base of the nail and found that a local application of histamine caused the capillaries to dilate and the blood to flow through them more rapidly. He interprets the increased rate of flow as an indication of a dilatation of the arterioles. There are, therefore, three distinct lines of evidence to show that the arterioles participate in the vasodilatation caused by histamine.

A moderate dose of atropin prevents the vasodilator action of acetylcholin. A dose of nicotine decreases the responsiveness of the vessels to a subsequent dose of nicotine. But our experiments have shown that neither of these substances is able to interfere with the vasodilator action of histamine.

We have taken occasion to inject atropin in doses of $\frac{1}{100}$ to $\frac{2}{100}$ grain into the blood stream going to the leg. Such doses usually produce transient vasodilatation. The plethysmographic tracings resemble those obtained by the use of histamine, but the response is not so regularly obtained. It is rather surprising that, while one dose of $\frac{1}{100}$ grain will prevent the action of a subsequent dose of acetylcholin, it does not prevent though it may somewhat impair the action of a subsequent dose of atropin. Four times the amount of atropin required to prevent the action of acetylcholin has no influence on the vasodilatation caused by histamine.

Since a dose of nicotine decreases the responsiveness of the vessels to a subsequent dose of the same toxin, the vasodilator reaction of nicotine cannot be obtained repeatedly on the same animal. But after repeated doses had rendered the vessels irresponsive to nicotine, the vasodilator action of histamine was still as good as ever.

The current view that the action of histamine is due to a "poisoning" of the capillary wall suggests the thought that after repeated doses these vessels would become irresponsive to chemical or neural stimulation. It was with the hope that we might throw the capillaries of the leg out of action and thus determine what part they played in the dilator action of nicotine and in antidromic vasodilatation that we began the intra-arterial injection of histamine. It should be remembered that by this method of injection the toxin acts on the capillaries of the leg in a concentration at least eight or ten times as great as on those in other parts of the body. But we found that after many doses of hista-

mine has been injected in this way, one following the other as soon as the blood pressure returned approximately to normal, the vessels continued to react to this toxin in the same way and to as great an extent as before. Nor was the reaction of the leg vessels to acetylcholin and nicotine in any way decreased by this prolonged exposure to rather concentrated doses of histamine. These statements hold true whether the doses of histamine were small ($\frac{1}{40}$ mgm.) or relatively large (2 mgm.), except that with larger doses a longer time was required for the blood pressure to return to normal.

Figure 1 records the action of 5 doses of $\frac{1}{5}$ mgm. of histamine separated by 3-minute intervals. It should be remembered that $\frac{1}{40}$ mgm. will produce a maximal vasodilatation. Each of the five doses of $\frac{1}{5}$ mgm. was therefore about eight times as great as that required to produce the effect recorded. Yet the last dose caused as much dilatation in the vessels of the leg as the first. This emphasizes the evanescent character of the dilatation which continues only while the histamine is present in appreciable quantities in the blood.

We expected to find that, after repeated injection of histamine into the blood going to the leg, the plethysmograph would show signs of a progressive and more or less permanent dilatation of the leg vessels. This did not occur ever when large doses of the toxin were used. Since in these experiments the toxin acted in much more concentrated form on the leg than on the rest of the body, we must conclude that, when a permanent condition of shock is produced by large doses of histamine, the prolonged low blood pressure is not due to a permanent paralysis of capillary tone produced by that toxin. Large doses of histamine doubtless produce, as Dale has suggested, a widespread capillary dilatation with an accumulation and stagnation of blood in these dilated vessels. This condition of circulatory failure is of itself irreparable and the defective circulation and lack of oxygen are the factors responsible for the continued capillary dilatation.

According to Dale and Laidlaw (8) the loss of plasma from the blood is one of the conspicuous features of histamine shock. Concerning this loss of plasma they say "It may be noted, in the first place, that it is probably another aspect of the same poisonous action of histamine on capillary endothelium. We may suppose that an intensification of the action which relaxes the normal capillary tone renders the endothelium abnormally permeable, so that it no longer retains the plasma." Even when repeated doses of 2 mgm. each have been introduced into the blood going to the leg the plethysmograph has usually shown no

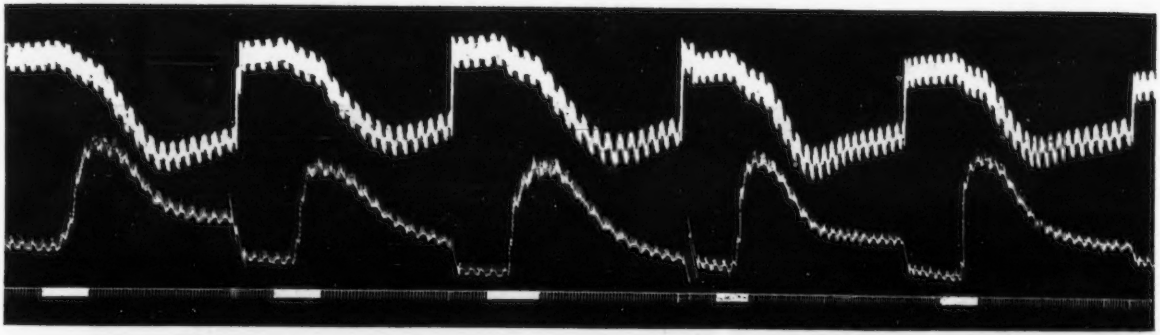

Fig. 1. Showing the effect on blood pressure (upper tracing) and leg volume (lower tracing), of the intra-arterial injection of 5 doses of histamine of $\frac{1}{2}$ mgm. each, separated by 3-minute intervals.

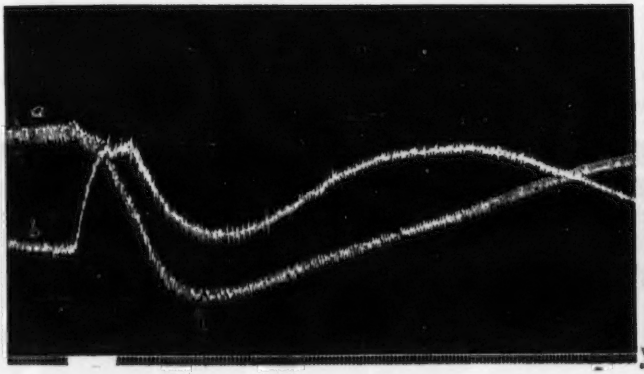

Fig. 2

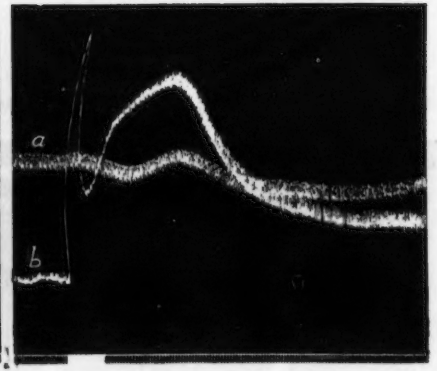

Fig. 3

Fig. 2. Showing the effect on blood pressure, *a*, and leg volume, *b*, of the intra-arterial injection of $\frac{1}{24}$ mgm. of histamine in a dog in which all the nerves going to the leg were degenerated.

Fig. 3. Showing the effect on blood pressure, *a*, and leg volume, *b*, of the intra-arterial injection of 1.5 mgm. of nicotine in a dog in which all the nerves going to the leg were degenerated.

evidence of the escape of fluid from the blood into the tissues. We are inclined to think that the increased permeability of the capillaries in histamine shock is a secondary result of the circulatory failure rather than an expression of changes in the endothelium directly due to the toxin. In connection with a study of the action of ether on the peripheral blood vessels we have had an opportunity to observe in plethysmographic tracings the swelling of the leg caused by an increased permeability of the capillaries (see fig. 3, *b*, on p. 324 of this number of the JOURNAL). Only once in our experiments with histamine have we obtained anything comparable to the swelling of the leg caused by the increased capillary permeability following intra-arterial injections of ether.

Since in these experiments all nerves going to the limb had been divided, the dilator reactions must have been purely local responses. They could not have been due in any part to the action of the central nervous system or sympathetic or spinal ganglia. There remained, however, the possibility of the stimulation of nerve endings in the vessel wall and to eliminate these another series of experiments was necessary.

SERIES II. *Experiments on dogs in which all the nerves to the left leg had degenerated.* In this series of aseptic operations the femoral and obturator nerves and the sympathetic plexus on the iliac artery were cut within the pelvis. The sciatic was divided in the upper part of the thigh. Ten to twelve days were allowed for degeneration. The experiments were then carried out on these animals in the same manner as in normal dogs. The results showed that the degeneration of all of the cerebrospinal and sympathetic nerve fibers going to the leg does not in any way impair the vasodilator reactions to be obtained from the intra-arterial injection of acetylcholin, nicotine, histamine or atropin. There is in fact a tendency for all of these reactions to be exaggerated and prolonged. The prolongation is usually more obvious than the increase in magnitude of the reaction (see figs. 2 and 3). Dale and Richards (4) have also noted the exaggerated response to acetylcholin and histamine after nerve degeneration. We agree with them that in all probability "the reactivity of normally innervated vessels is restricted by the effect of tonic impulses from nerve centers; only when these are cut off, and when the stimuli aroused by nerve section and the earlier processes of degeneration have passed off can the contractile elements in the blood vessels exhibit their unhampered response to these drugs."

A curious feature of the nicotine reactions in all of the dogs of this series was a sudden sharp rise in the plethysmographic curve which preceded the vasodilator reaction (fig. 3). This did not occur in normal dogs and was probably due to a stimulation and contraction of the denervated and atrophying skeletal musculature.

While this series of experiments shows conclusively that the vasodilator reactions produced by acetylcholin, nicotine, atropin and histamine are not dependent on the integrity of the endings of nerve fibers from the cerebrospinal or sympathetic systems, it does not exclude the possibility that some of these substances may act on a hypothetical autonomous nerve net in the arterial wall. But in one of the preceding papers of this series (1) it has been shown that it is not very probable that any such autonomous nerve net exists on the blood vessels.

CONCLUSIONS

When histamine is injected directly into the arterial blood stream going to the leg, plethysmographic tracings show that:

1. In doses of 1 mgm. or less histamine causes a transient vasodilatation as evidenced by swelling of the leg and increased amplitude of the pulse. The increase of the pulse waves indicates that the arterioles as well as the capillaries are involved in the reaction.

2. The action of histamine is of short duration, persisting only as long as the toxin continues to circulate in the blood. The blood vessels recover their normal tone very quickly. The idea that it "poisons" the endothelium must be modified by the further statement that the vessels recover very quickly and completely. Histamine does not of itself seem to cause an increased permeability of the capillary walls.

3. The action of histamine is not prevented by previous doses of atropin or nicotine.

4. The vasodilator reactions caused by histamine, acetylcholin, atropin and nicotine are purely peripheral responses and are not in any way dependent on the vasomotor center nor on the spinal nor sympathetic ganglia.

5. After the degeneration of all cerebrospinal and sympathetic fibers going to the leg the vasodilator reactions produced by histamine, acetylcholin, atropin and nicotine are even more pronounced and of longer duration than in normal animals. Hence these substances do not act on the endings of the cerebrospinal or sympathetic nerve fibers.

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VASODILATOR MECHANISMS

V. The Intra-Arterial Injection of Ether

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Relatively little has been written in recent years on the pharmacology of ether. But it is known that during the first two stages of anesthesia the blood pressure rises slightly due to struggling and to reflex excitation of the vasomotor center as a result of irritation of the respiratory passages. Possibly ether in small amounts may stimulate the vasomotor center. When perfused through the peripheral blood vessels it is said to cause vasodilatation. If the anesthetic is pushed beyond a certain point there is a fall in blood pressure due in part to depression of the vasomotor center. In the stage of complete anesthesia the pulse is slower and weaker than normal, and this cardiac depression increases with the depth of anesthesia (Cushing).

At the end of an experiment on the effect of intra-arterial injection of histamine it occurred to us that it would be easier to kill the animal by injection of ether into the blood stream than by increasing its concentration in the lung. Accordingly we injected 6 cc. of ether into the aorta through the central end of the right iliac artery. We were surprised to find that the blood pressure instead of falling was greatly increased and remained high for many minutes. When it became evident that this was the usual effect of the intra-arterial injection of relatively large doses of ether we felt justified in digressing from the problems presented by the vasodilator mechanisms to consider this peculiar phenomenon.

Medium sized dogs were used except in a few experiments. The right iliac artery had previously been brought out through an opening in the flank; and drugs injected centrally through this vessel reached the bifurcation of the aorta and were carried with the blood stream through the left iliac to the left leg and left side of the pelvis. Usually

the injection of ether was made at the end of a long experiment during which repeated doses of acetylcholin, atropin and histamine had been administered through the iliac artery and the blood pressure was considerably below the original level. In fact the most impressive results can be obtained with animals in which for one reason or another the blood pressure has become stabilized at a rather low level. It also appeared that better results were obtained if both vagi had been cut, since the sudden increase in the amount of ether circulating in the blood seemed to stimulate the cardio-inhibitory center. In most cases the animals had been receiving ether through an ether bottle and tracheotomy tube for an hour or more and had been given 60 mgm. of morphine at the start. "Anesthetic ether" was used for the intra-arterial injections and two different brands were employed. This precaution was taken in order to exclude the possibility that the pressor response was due to some impurity.

In each case a tracing was made of the carotid blood pressure and in many, though not all, a plethysmographic record was taken of the volume of the left leg. The best results were obtained with doses of from 3 to 6 cc., according to the size of the dog.

Intravenous injections of these large doses of ether in animals already fully anesthetized cause a sudden fall in blood pressure and almost immediate death of the animal. Even 1 cc. injected intravenously in a dog weighing 10 kilos caused a marked fall in blood pressure, while the same amount administered through the iliac artery produced a slight rise (fig. 3). The plethysmographic record shows that the first effect of the ether when injected through the artery was a constriction of the arteries of the leg as shown by decreased amplitude of the pulse and a decreased volume of the leg. This primary constriction was, however, soon followed by dilatation as evidenced by the increased amplitude of the pulse and a swelling of the leg. But without doubt the steadily progressing increase in volume is not all due to vasodilatation.

A good illustration of the action of a larger dose of ether when injected into the arterial blood stream is furnished by figure 1. The dog from which this tracing was taken weighed 8.5 kilos and had been under ether anesthesia for an hour and a half. During the 50 minutes immediately preceding it had received 5 doses of 2 mgm. each of histamine. The blood pressure which had originally stood at 96 had been reduced by the histamine and by the prolonged experiment, in which no effort had been made to conserve the body heat. For some time preceding the injection of the ether the blood pressure had stood

Fig. 1.

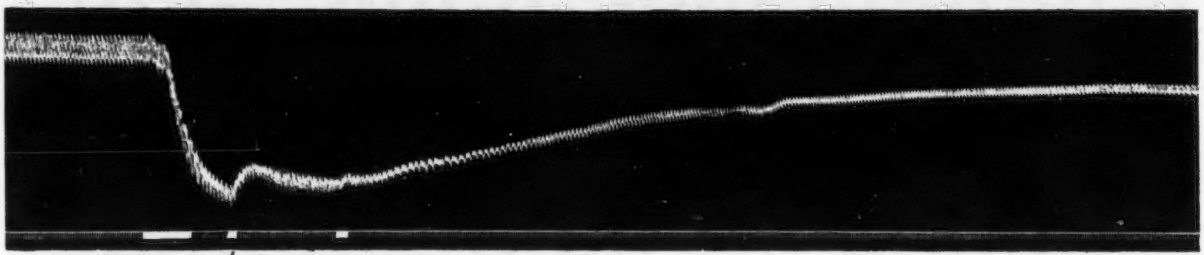

Fig 2

Fig. 1. Showing the effect, in a dog weighing 8.5 kilos, of the injection of 6 cc. of ether into the aorta through the right iliac artery. The upper tracing represents the carotid blood pressure; the lower tracing, the volume of the left leg.

Fig. 2. Tracing of the carotid blood pressure of a dog weighing 10.5 kilos, showing the effect of intra-arterial injection of 9 mgm. of histamine, *a*, followed immediately by 6 cc. of ether, *b*, and 3 cc. of salt solution, *c*. The ether counteracted the histamine and caused the blood pressure to return toward normal much quicker than it would have done if the ether had not been given.

at 66. The injection of 6 cc. of ether caused a slight rise in blood pressure during the few seconds required for the injection due to the vaporization of the ether in the warm blood. This effect was transitory as the ether quickly went into solution. A few seconds after the injection the pressure began to rise rapidly and soon reached 168, which was considerably higher than the original pressure. From this high point the blood pressure fell very gradually showing that the pressor effect produced by the injection persisted with decreasing strength for a considerable time. Both curves in figure 1 show an increased amplitude of the respiratory waves at the time of and immeditately following the injection. The increased depth of respiration was probably caused by the excitation of the respiratory center reflexly through the sensory nerve endings in the vessel walls.

The plethysmographic tracings in figures 1 and 3 show irregularities at the time of, and immediately following, the injections due to the exaggerated respiratory movements. Both tracings show a primary shrinkage, due to vasoconstriction, followed by a swelling of the leg which, judging from the increased amplitude of the pulse, must be in part due to vasodilatation. The expansion of the leg caused by the intra-arterial injection of 1 cc. of ether is illustrated in figure 3, *b*. The swelling progressed at the rate indicated in the tracing for 25 minutes and continued with a decreasing rate for 30 minutes more. That is to say, the leg continued to increase in volume for nearly an hour. Figure 1 shows a similar reaction following the injection of 6 cc. of ether. In all our experiments, in which volume was recorded, the swelling of the left leg, through the vessels of which ether had passed in concentrated form, was at least as conspicuous as that shown in figure 1 and continued to progress for from 30 to 60 minutes. Although vasodilatation undoubtedly occurs as evidenced by the increased amplitude of the pulse waves it is not probable that this alone could explain the swelling which progresses at so uniform a rate over so long a period. It seems necessary to assume that the ether has caused an increased permeability of the capillary walls and an excessive transudation of fluid into the tissues.

Since the rise in blood pressure was more evident when the original pressure was low we found it desirable to first reduce the pressure by the injection of histamine. When injected into the arterial blood stream ether is able to counteract the effect of histamine, as shown in figure 2. The dog from which this tracing was taken weighed 10.5 kilos. Immediately following the intra-arterial injection of 9 mgm. of

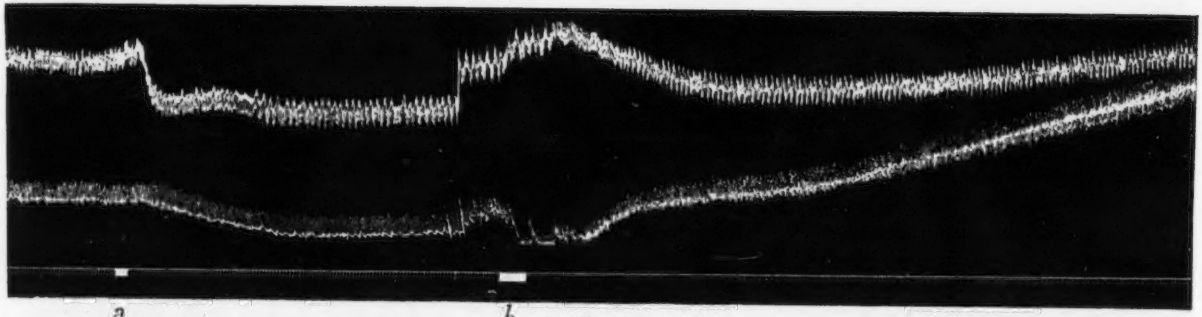

Fig. 3.

Fig. 4.

Fig. 3. Showing the effect, on a dog weighing 9.5 kilos, of 1 cc. of ether when injected into the jugular vein, *a*, and when injected into the aorta through the right iliac artery, *b*. Upper tracing, carotid blood pressure; lower tracing, volume of the left leg.

Fig. 4. Carotid blood pressure tracing, showing the effect on a dog weighing 3 kilos of the intravenous injection of 20 cc. of blood from the inferior vena cava of another dog which had previously received 3 cc. of ether through the iliac artery; *a*, 20 cc. of blood and, *b*, 3 cc. of normal salt solution injected into the external jugular vein; *c*, 20 cc. normal salt solution injected into external jugular vein.

histamine, an amount which should have produced a very prolonged low blood pressure and perhaps permanent histamine shock, 6 cc. of ether were injected into the iliac artery. The fall in blood pressure caused by the histamine was at once arrested and in about a minute there began a rise which continued until the pressure reached nearly the original level.

During the stage of increased blood pressure following the injection of ether the vasodilator action of histamine is decreased. For example, in one dog before the injection of ether, 0.4 mgm. of histamine caused a drop of 50 per cent in the blood pressure while immediately after the ether 8 mgm. of histamine caused no drop at all. From the evidence presented in this and the preceding paragraph it is evident that, if the low blood pressure in histamine shock is due to capillary dilatation, the intra-arterial injection of ether must in some way counteract this capillary dilatation.

Similar reactions were obtained in cats by injecting ether toward the aorta in the right carotid artery while a tracing of the blood pressure was being taken from the left carotid. In one cat, weighing 3.6 kilos and having at the start a blood pressure of 120 mm., the administration of 5 mgm. of histamine in 0.5 mgm. doses extending over a considerable time caused the blood pressure to fall to 40 mm. Immediately following the injection of 2 cc. of ether centrally through the right carotid artery the pressure rose rapidly and at the end of 2 minutes reached 150 mm., and then very gradually fell again reaching 52 mm. at the end of 17 minutes. In another cat, weighing 3.4 kilos and having an initial blood pressure of 124 mm., repeated doses of histamine reduced the pressure to 68 mm. but it rose to 168 mm. after the intra-arterial injection of ether. In both of these experiments the pressure reached levels considerably higher than that at which it stood at the beginning of the experiment. In all cases where ether was injected centrally in the right carotid, respiration ceased immediately and artificial respiration was started at once. The drug was no doubt distributed chiefly through the branches of the right subclavian artery and reached the medulla in concentrated form through the vertebral. Occasionally, but not usually, following injection of ether through the iliac artery artificial respiration was required. In all experiments preparation had been made for artificial respiration so that it could be started without delay and in no case could there be any question of an asphyxial rise.

The most probable explanation of this peculiar action of ether seems to be that when passing through the capillaries in concentrated form

it in some way changes the permeability of their walls so that a pressor substance contained in the endothelium or in the surrounding tissues passes into the circulation in greater than normal volume. An increased permeability of the capillaries for fluid passing from the blood into the tissues is, we believe, demonstrated by the plethysmographic tracings.

Assuming that this action of the ether is primarily on the capillary walls it is easy to understand why repeated previous doses of histamine favor the development of the pressor reaction. Histamine is supposed to cause capillary dilatation: so that in a field, where normally only a few capillaries would be patent, the majority become opened up. Under these conditions the ether, when injected intra-arterially, must pass through a widely dilated capillary bed. It would have opportunity to act in concentrated form on a relatively large amount of capillary endothelium and a considerable amount of it would be taken up by the tissues. In the ether injections which we have made in dogs, having a normal blood pressure, i.e., in dogs in which there was the normal degree of vasoconstrictor tone, we have not secured a pressor response but often instead a fall in pressure. In these cases we assume that the ether was forced rather rapidly through a more limited number of capillaries and more of it was carried with the venous blood back to the heart.

In order to test the theory that an increased permeability of the capillary wall facilitates the entrance of a pressor substance into the blood stream from the tissues, we resorted to transfusion experiments. In dogs under ether anesthesia the abdominal cavity was opened by a median incision; the right iliac artery was prepared for injection toward the aorta; the right common iliac vein was ligated and a cannula tied into its central end so that blood could be removed through it from the vena cava; and a ligature was passed under the vena cava 1 cm. above the junction of the iliac veins. Three doses of 1 mgm. each of histamine were injected into the aorta through the right iliac artery in order to fully dilate the capillaries of the left leg and pelvis. The last dose of histamine was followed at once by 3 or 4 cc. of ether and then an equal amount of salt solution to wash all of the ether from the blind end of the artery into the circulation. Two minutes after the injection of ether the ligature around the vena cava was tied, it being assumed that 2 minutes was sufficient for the blood laden with an excessive amount of ether to have been displaced from the leg and its veins by fresh blood. After ligating the vena cava, the blood, coming from the

left leg, was removed through the cannula in the right internal iliac vein and mixed with enough 10 per cent sodium citrate to make a 0.5 per cent citrate solution. The blood obtained in this manner was then injected intravenously into another animal which had previously been prepared for a record of the carotid blood pressure and which was receiving ether in the usual way through an ether bottle and tracheal cannula.

In the first three of these transfusion experiments the recipient was in each case a cat which had received 3 mgm. of histamine in divided doses shortly before the transfusion. In each case injection of 10 cc. of normal dogs' blood into the cat's jugular vein caused only a negligible rise in blood pressure, but when 10 cc. of blood, taken from the dog's vena cava 2 minutes after the injection of ether, were injected into the cat's jugular vein there occurred a considerable and rather prolonged pressor response.

In four other transfusion experiments the recipients were small dogs weighing 3 or 4 kilos. In one of these the experiment was a failure because of clotting of the blood. In two others the recipient's blood pressure, which was high at the start, was reduced to a low level by histamine before the transfusion was made. In both of these the intravenous injection of 20 cc. of blood from the vena cava of the donor caused a rise in pressure which could not be reproduced by the injection of an equal amount of salt solution. In the fourth experiment the recipient's blood pressure was low at the start and no histamine was administered.

In this fourth transfusion experiment the recipient dog weighed 3 kilos and had an initial blood pressure of 56 mm. The cause for this low pressure was not ascertained but it was not due to excessively deep anesthesia nor to hemorrhage. The pressure maintained itself steadily at this low level until there was injected into the jugular vein 20 cc. of blood freshly drawn from the inferior vena cava of another dog which had received 3 cc. of ether through the iliac artery. Following the transfusion the pressure rose from 56 to 92 mm. and was maintained at a high level for a considerable time (fig. 4, *a*). After the pressure had fallen again to 60 mm., 20 cc. of normal salt solution were injected intravenously without causing an appreciable rise in pressure (fig. 4, *c*). Two subsequent injections of blood from the vena cava of the donor caused the same slow and rather prolonged rise in blood pressure, but after neither did the pressure rise as high as after the first injection.

Since during the 2 minutes which elapsed after the injection of ether before the ligation of the superior vena cava the excess of ether must have been completely washed out of the vessels of the leg by fresh blood, it seems most reasonable to attribute the pressor action of this blood to the presence in it of some substance absorbed from the tissues of the leg through capillaries whose permeability had been increased by ether.

In order to determine whether this hypothetical pressor substance acts on the vasomotor center or elsewhere the spinal cord was cut in a dog at the level of the first thoracic segment. The blood pressure, which was recorded from the left carotid artery, fell to 42 mm. Six cubic centimeters of ether were then injected toward the aorta in the right carotid artery and artificial respiration instituted. The blood pressure rose from 42 to 108 mm. within 2 minutes after the injection and very gradually fell again reaching 48 mm. at the end of 10 minutes. This experiment shows that the pressor response to the intra-arterial injection of ether is not due to the stimulation of the vasomotor center. The hypothetical pressor substance probably acts either on the vessel walls or on the endings of the vasoconstrictor nerve fibers.

While this paper was in press, there appeared in the Archives of Surgery an important article by Cattell dealing with the action of ether on the circulation in traumatic shock. A comparison of his results with our own suggests the possibility that even during an ordinary anesthetic some of our hypothetical pressor substance may be mobilized and constitute an important factor in maintaining the blood pressure at the normal level in spite of the depressing action of ether on the heart.

CONCLUSIONS

1. When blood containing a large amount of ether passes through the vessels of a limb it causes, first, a vasoconstriction and, later, a vasodilatation and a swelling of the leg. The swelling continues to progress for a long time, sometimes for an hour or more, and is probably caused, for the most part, by an escape of fluid from the blood due to an increased capillary permeability.

2. In an animal having a low blood pressure as a result of a generalized vasodilatation such as occurs in spinal shock or histamine poisoning, the intra-arterial injection of ether causes a pronounced and prolonged rise in blood pressure.

3. The blood returning from a limb whose vessels have been subjected to the action of ether in rather concentrated form contains a pressor

substance and causes a rise in blood pressure when injected into another animal.

4. The theory is suggested that ether when present in the blood in rather concentrated form increases the permeability of the capillaries and allows a pressor substance to enter the blood from the tissues in greater than normal amount.

EXTRACTION OF THE ANTI-DIABETIC SUBSTANCE (INSULIN) BY PERFUSION OF THE PANCREAS

I. The Influence of Neutral or Alkaline Perfusates on the Respiratory Metabolism of Depancreatized Animals

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The positive results obtained by Murlin and Kramer (1) from the administration to depancreatized dogs of pancreatic extract made in acid solution together with glucose and a small amount of alkali encouraged the hope that it might be possible to wash out the anti-diabetic substance by perfusion of solutions through the blood vessels of the pancreas. The respiratory quotient of totally depancreatized dogs was raised several times from 0.69 and 0.70 to 0.79 and once to 0.87, showing in the light of numerous controls with alkali alone that the power to oxidize sugar had been restored in part, and the dog (no. 51) survived the operation of pancreatectomy with the help of this treatment at least a week longer than other animals not so treated. There was also a dynamic action from the glucose. Clark (2) had devised a method of perfusion of the pancreas and was able to demonstrate improved utilization of sugar by the heart of a diabetic animal when the perfusate was circulated through it. We determined therefore to try the effect of perfusates made in the same manner on the entire animal. This work was undertaken as part of a program which was to extend over at least two years, the remainder of the plan contemplating preparation of extracts in acid media in exactly the same manner as Murlin and Kramer had prepared such extracts in 1913. The work was begun in October, 1921, and has continued uninterruptedly to the present time. It was unknown to us that workers at the University of Toronto were engaged on the problem until shortly before the December 1922 meetings at New Haven at which were presented the first results of Banting and Best from Macleod's laboratory.

Knowing that the ultimate criterion of relief from the diabetic condition must be the demonstration of actual combustion of sugar we determined to strike for this objective at once. A respiration apparatus capable of giving reliable respiratory quotients was assembled and proved by alcohol checks. Animals made totally diabetic by removal of the pancreas gave typical quotients for this condition, after which the administration of alkaline perfusates was found in several instances to raise the quotient quite perceptibly. In all but one of the experiments reported here cats were used as subjects. One experiment with an alkaline perfusate of pig's pancreas upon a diabetic dog is reported. A later series *where acid perfusates were used* upon depancreatized dogs, which are more favorable for observations on the blood sugar and D:N ratio and not less so for the respiratory exchange will constitute the subject matter of the next paper.

METHOD OF PERFUSION. *Apparatus.* The apparatus used for perfusion is essentially like that used by Clark. Certain minor modifications were made to suit our special requirements.

Before use the individual parts: fluid chamber, bead chamber, pancreas chamber, tubes with valves, manometer tube and syringe, were placed in double walled cloth sacs and sterilized in an autoclave at a pressure of 15 pounds for 15 minutes. Parts of the apparatus which would be exposed after removal from the cloth sacs, such as the connectors for the syringe and manometer tube, were separately covered with double-walled cloth tubing. In this way it was possible to set up the entire apparatus without contaminating any of its parts.

The perfusion apparatus was assembled in a large incubator 28 inches × 18 inches × 14 inches kept at body temperature by means of an electric heating element controlled by a thermostat. Surrounding the incubator was a scaffolding of wood on the top of which was located a motor, speed reducing gears, eccentric wheel and micrometer screw for adjusting the length of the piston stroke. Three speeds were available giving 8, 14 and 22 strokes per minute. By means of the eccentric and micrometer adjustment any length of stroke could be obtained from zero to the maximum allowed by the piston of the syringe. Mounting the driving apparatus upon the wooden scaffolding independent of the incubator served to protect the thermostat from constant vibration.

The entire driving apparatus was independent of the incubator except for the shaft connecting the eccentric with the syringe plunger. This shaft was passed through a ventilating hole in the top of the

incubator. The length of stroke could be varied as necessity arose, during the perfusion, without disturbing the temperature of the chamber since all adjustments were made from the outside. A switch near the front of the apparatus made it possible to control the pump while it was under observation.

Inside the incubator the apparatus was supported by means of three clamps all attached to a shelf fastened transversely across the incubator chamber. A large wooden clamp supported the barrel of the syringe throughout its entire length. A second wooden clamp supported the bead chamber. A third clamp of metal supported the glass tubing and valves. The jaws of these clamps were opened to admit the parts and then firmly closed with adjusting screws so as to give rigid support to all parts of the apparatus.

Outside the incubator was a large oxygen cylinder, a wash bottle containing concentrated H_2SO_4 and a second wash bottle containing glass beads and sterile water. A small rubber tube extended from the second wash bottle through a ventilating tube at the lower side of the incubator to the inner chamber where it was connected with the apparatus.

After the completion of a perfusion the apparatus was washed with soap and water, rinsed with water, and filled with 95 per cent alcohol which was pumped through the apparatus at frequent intervals.

Perfusion fluid. The perfusion fluid was made according to the following formula:

Sodium chloride	9.00 grams
Potassium chloride	0.42 grams
Calcium chloride	0.24 grams
Distilled water to	1,000 cc.

In several instances glucose was added to 0.5 and 1.0 per cent respectively. The fluid was placed in a flask and sterilized in an autoclave to 15 pounds pressure for 15 minutes. The solution was cooled to body temperature and 0.20 gram of sodium bicarbonate added. All the perfusions reported in this paper were made with weakly alkaline solutions. The perfusion fluid was sterilized in 500 cc. volumetric flasks and the volume lost during sterilization was restored, after cooling, with sterile water. The perfusion fluid and assembled apparatus was kept in the incubator several hours before a perfusion was done in order to have all at a uniform temperature.

Preparation of the pancreas. The pancreas was prepared for perfusion under aseptic conditions in a well equipped and sanitary opera-

ting room, as follows: The splenic end of the organ is first separated from its attachments just as in ordinary pancreatectomy, all blood vessels being ligated. The dissection having been carried up to the intestine, the splenic end is brought through a slit in the duodenal

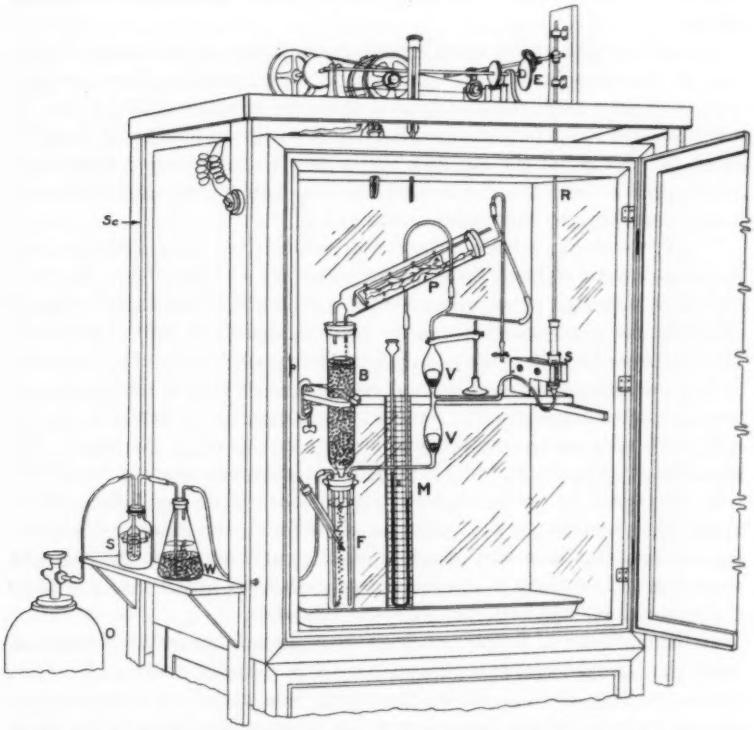

Fig. 1. Perfusion apparatus. *F*, fluid chamber; *B*, bead chamber; *P*, pancreas chamber; *V*, valves; *S*, 10 cc. syringe used as a pump; *R*, piston rod from driving mechanism; *E*, eccentric for regulating stroke; *M*, manometer for recording perfusion pressure; *O*, oxygen tank; *S*, sulphuric acid; *W*, sterile water; *Sc*, scaffolding of wood supporting motor and reducing gears.

mesentery to the right side of the intestine and the dissection continued until the superior pancreatico-duodenal vessels are clearly exposed. At this point the entire splenic end of the organ is removed after coarse ligation with gauze. The uncinate process is likewise

removed; for, like the splenic end, it has in the cat and dog an independent blood supply. The point of separation can be fairly judged by the distribution of the blood vessels. There remains about one-half the weight of pancreas but hardly more than one-third its length which can be perfused through the superior pancreatico-duodenal artery.

Several methods were tried for the cannulation of this artery; insertion of the cannula with the pancreas in situ, insertion after the pancreas had been removed and floated in sterile Ringer's solution, and in a few cases direct insertion after removal but without floating in Ringer's solution. Either of the last two methods is to be preferred where it is not desired to sacrifice the animal, for the respiratory movements are quite disturbing to successful cannulation.

Clark, we think, gives a wrong impression in his statement that the pancreas can be isolated from the intestine in a few minutes. In order to insure thorough penetration of the perfusate it is necessary to ligate carefully all anastomosing vessels between pancreas and duodenum, but this is well worth while as many primary leaks are thereby avoided.

In a few cases the pancreas and contiguous portion of the duodenum were removed together, the resected duodenum being firmly closed at each end with sutures to prevent leakage of intestinal contents. We question whether it would be possible to preserve aseptic conditions with the open intestinal segment strung on a glass rod immediately above the pancreas in the perfusion chamber in the manner described by Landes, Garrison and Moorehead (3), particularly as the segment was observed by them to exhibit active peristalsis which would tend to discharge duodenal contents into the chamber.

In the perfusion of the pig pancreas the organ was immediately removed from the slaughtered animal and placed in a sterile thermos food jar. After reaching the laboratory the organ was placed in a sterile pan, the cut ends of arteries ligated, and the cannula inserted into the main artery. A small quantity of physiological saline injected with a syringe served to locate the cut ends of arteries. Under favorable conditions it was possible to transport the pancreas across the city by automobile, complete the dissection, ligation of vessels and insertion of the cannula, and begin the perfusion within 45 minutes after the removal of the organ from the pig.

Perfusion pressures and volume. It was not found feasible to adopt any one pressure for all cases. Owing to the great variation in the size of the organs (cat 8 grams to pig 180 grams), the impossibility of

ligating many of the small arteries and the variation in the amount of blood left in the organs at the time of removal, it was found desirable to adapt the pressure and rate of perfusion to the organ in question rather than to adopt an arbitrary rate and pressures to be used in all cases.

When the perfusion was satisfactory the pancreas would immediately become pink, swollen, spongy in appearance and would show a systolic pulsation with each stroke of the pump. In the most successful perfusions both systolic and diastolic pressures were high. The pressures used in the perfusion of two cats' pancreases were systolic 80 mm. Hg, diastolic 30, rate 8.5 cc. per minute; systolic 110, diastolic 100, rate 7 cc. per minute. Pressures for dogs were as follows: systolic 180, diastolic 140, rate 39.6 cc. per minute; systolic 106, diastolic 78, rate 19 cc. per minute.

Changes in pressure during the interval of perfusion were common. Usually after the first 10 or 15 minutes the pressures would fall slightly due either to the complete washing out of blood or to the development of leaks from small vessels. In the case of perfusion of the pancreas with a portion of duodenum the pressure would rise coincident with the distention of the duodenum with fluid. In one such experiment the pressures rose from systolic 140, diastolic 120, at the beginning to systolic 260, diastolic 120, with a rate of 24 cc. per minute, at the end of the perfusion.

Injection of pancreas. After the conclusion of the perfusion a syringe full of India ink (1:3) was injected through the cannula with the pancreas in situ. Subsequent section of the organ with a scalpel served to show the course of the perfusate. In nearly every case the penetration involved all parts of the perfused mass.

METHOD OF DETERMINING RESPIRATORY QUOTIENTS. A modified Jaquet (4) method has been used in determining the respiratory quotient. The essential features of the Jaquet method consist of: *a*, a respiration chamber in which the subject is confined; *b*, a continuous stream of air drawn through for ventilation, the total volume being measured by means of a meter, and *c*, a means of taking a continuous sample of air for analysis. The respiration chamber (fig. 2) which we have employed in this series is constructed of 14-ounce copper and measures 29.5 × 75.4 × 15.0 cm. in the rectangular portion. The bottom half is the sector of a cylinder with a radius of 15 cm. The volume including the lid is 63.5 liters. The chamber is closed at the top by means of a lid containing a glass panel, the edge fitting into a water seal 6 cm. in depth.

Air enters the chamber at one end near the top and leaves at the other end near the bottom. The animal rests upon a wire net supported 10 cm. above the bottom of the chamber. The current of air is drawn in from outdoors through a rubber tube 2 cm. in diameter

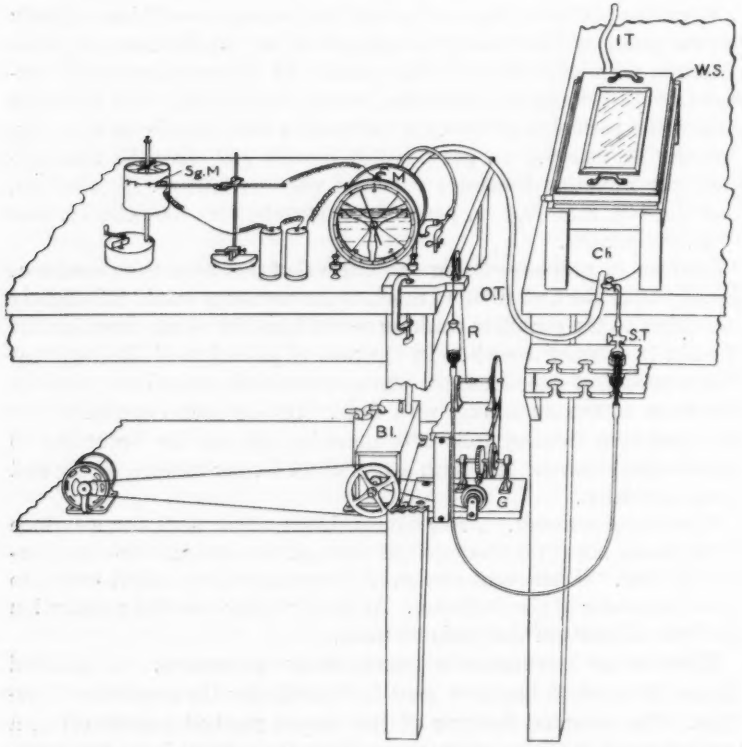

Fig. 2. Respiration apparatus. *Ch*, chamber for animal; *Gl*, glass panel in lid; *W.S.*, water seal; *I.T.*, inlet tube; *O.T.*, outlet tube; *S.T.*, sampling tube; *R*, reservoir of mercury; *G*, gears for lowering mercury reservoir at a rate proportional to rate of ventilation; *Bl*, Crowell blower; *M*, Bohr meter for measuring total ventilation; *Sg.M.*, signal magnet for recording revolutions of meter.

which perforates the window sash and projects several feet beyond. The corner of the building is so sheltered that no special precaution against wind effect has been found necessary.

The air current is actuated by a Crowell blower driven by a motor and mounted on a Benedict universal respiration table. Only a part of the air necessary to feed the blower running at the lowest speed possible is drawn through the chamber, the remainder entering through the other limb of a T connection from the room. The chamber air is first drawn through a Bohr meter of 5 liters capacity where it is measured automatically by means of an electric signal operated at each revolution of the indicator.

ALCOHOL CHECKS. The apparatus has been tested at frequent intervals by burning alcohol in the chamber. Following are a few typical examples of the accuracy of the method.

DATE	CHAMBER AIR		R.Q.		PER CENT ERROR
	Per cent CO_2	Per cent O_2			
1921					
December 10	2.09	17.93	0.667		
	2.37	17.54	0.641	0.649 av.	−2.5
	2.07	17.97	0.639		
December 23	1.766	18.46	0.660		
	1.852	18.34	0.653	0.650 av.	−2.4
	1.904	18.21	0.637		
December 31	0.221	20.71	0.659		
	0.690	19.98	0.660	0.664 av.	−0.3
	0.732	19.96	0.675		
1922					
January 6	0.371	20.44	0.636		
	0.370	20.45	0.663	0.655 av.	−1.7
	0.400	20.46	0.667		
January 24	0.860	19.71	0.669		
	0.997	19.51	0.662	0.664 av.	−0.3
	0.990	19.51	0.660		
March 17	1.550	18.72	0.638		
	1.569	18.73	0.652	0.647 av.	−2.8
	1.848	18.31	0.652		
April 1	0.664	20.03	0.660		
	0.513	20.21	0.657	0.664 av.	−0.3
	0.583	20.11	0.674		

The air sample for analysis is taken from the exit tube at a point 9 cm. from the chamber. It may be taken continuously over a period of 10 to 15 minutes. This is accomplished by lowering the mercury leveling bulb at the appropriate rate by means of a series of gears (*G*, fig. 2) attached to the driving pulley of the blower. With any given inlet of air from the chamber the ratio of sample to total ventilation is therefore constant.

Air is analyzed by means of the Henderson (5) and Bailey (6) modification of Haldane's instrument.

For the present series of observations the total ventilation and total metabolism has been disregarded, the respiratory quotient only being desired as a criterion of combustion of glucose.

Checks on the analysis of outside air have been fairly satisfactory as may be seen from the following.

DATE	OUTSIDE AIR	
	Per cent CO_2	Per cent O_2
1921		
December 30	0.03	20.917
	0.03	20.928
1922		
January 3	0.03	20.912
	0.03	20.950
	0.03	20.97
	0.02	20.885
January 6	0.03	20.93
	0.03	20.945
	0.03	20.96
April 6	0.02	20.93
	0.03	20.93

Even better checks on the accuracy of the method are obtained by the diabetic animal itself. Lusk (7), following Magnus-Levy (8), has calculated the respiratory quotient for protein in diabetes where the G:N ratio is 3.65 to be 0.632, and for fat where 36 grams B oxybutyric acid is formed from 100 grams fat to be 0.669. Respiratory quotients in severe diabetes in man (9) have been found as follows:

G:N ratio	*R. Q.*
3.5	0.697
3.97	0.687

The totally depancreatized dog yields similar quotients. In previous papers (10) values like the following were frequently found when the dog was subsisting on a diet of lean meat or meat and lard.

DATE	DOG	R.Q.	DIET
July 18, 1914	3	0.69 0.68	Meat only
July 6, 1916	54	0.71 0.68	Meat only
December 4, 1915	45	0.72 0.68 0.69 0.72	Meat only
May 20, 1916	51	0.68 0.71	Meat and lard
May 29, 1916	51	0.66 0.68	Meat and lard
June 26, 1916	53	0.67 0.69	Meat only

EXPERIMENTAL DATA. Cat 10. Weight 3.9 kgm., operated February 25, 1922. Hédon technique. Time of operation, 40 minutes. Extract made from pancreas. February 27, cat in good condition; eats fish, drinks well, bowels open, temperature normal.

Perfusate no. 4 for cat 10, March 2, made as follows: Cat's pancreas; 250 cc. sterile Ringer's solution used. Perfusion started 3:40 p.m., continued until 7:55 p.m. Rate 8½ cc. per minute. Perfusate tinged with blood for first 15 minutes, thereafter clear. Systolic pressure 80 mm. Hg, diastolic 30. Total perfusion 2400 cc. or 8 times through the pancreas. Injection with India ink showed traces to extreme end of organ. Near the cannula a small portion did not receive the ink due to rupture of a small branch of the artery. Microscopic examination showed capillaries injected.

Perfusate no. 5 used with cat 10 on March 4, prepared from pancreas and contiguous portion of duodenum of dog. Perfusion begun at 3:25 and continued until 4:25 p.m. Systolic pressure 140, diastolic 120 at beginning; later rose to 260 mm. systolic and 120 diastolic. Rate 24 cc. per minute. Perfused through pancreatico-duodenal vein. Injection of India ink showed perfusion of all parts of the pancreas but none in mucosa of duodenum. *Indicates that anastomosis between pancreas and intestine involves outer coats only.*

TABLE 1

Cat 10. Influence of perfusate of cat and dog pancreas

DATE	TIME	TREATMENT	NUMBER OF ANALYSIS	INSPIRED AIR		EXPIRED AIR		R.Q.
				Per cent CO_2	Per cent O_2	Per cent CO_2	Per cent O_2	
1922			646	0.03	20.84			
February 28	9:15 p.m.		647			0.798	19.88	0.695
	9:45		648			1.07	19.53	0.712
	10:20		649			0.865	19.74	0.658
	11:30		650			0.655	20.07	0.694
						Average		0.695
	4:50	30 gm. dextrose by stomach tube						
	5:40	20 gm. sucrose by stomach tube						
	5:50	Placed in chamber						
	6:30		660			0.565	20.18	0.676
	6:50		661			0.515	20.255	0.683
	7:10		662			0.523	20.27	0.715
						Average		0.691
	7:55	30 cc. perfusate of cat's pancreas by vein						
	8:15		663			0.547	20.24	0.718
March 2	8:35		664			0.5295	20.25	0.703
	10:13							
	10:25	67 cc. perfusate no. 4 of cat's pancreas by vein						
	10:35	20 gm. sucrose in 100 cc. water by stomach tube						
	10:55		666			0.6568	20.125	0.750
	11:15		667			0.7975	19.945	0.748
	11:20	Cat passed large amount of fluid by bowel						
		Chamber cleaned up						
	11:30	Returned to chamber				0.474	20.36	0.751
	10:35 a.m.		670			1.110[1]	19.47	0.703
	11:50		672			0.453	20.33	0.671
March 4	12:05 p.m.		673			0.452	20.34	0.681
						Average		0.685

TABLE 1—*Concluded*

DATE	TIME	TREATMENT	NUMBER OF ANALYSIS	INSPIRED AIR		EXPIRED AIR		R.Q.
				Per cent CO_2	Per cent O_2	Per cent CO_2	Per cent O_2	
1922								
March 6	10:00 a.m.		674			0.586	20.16	0.686
	12:10 p.m.		678			0.504	20.285	0.696
	5:25 (—)							
	5:55	35 cc. perfusate no. 5 of dog's pancreas and duodenum by vein						
	6:00	20 gm. sucrose in 50 cc. water by stomach tube						
	6:15		679			1.019*	19.685	0.760
	6:45		680			0.598	20.19	0.738
	7:10		681			0.504	20.31	0.738
	7:50		683			0.516	20.295	0.735
	8:10		684			0.525	20.30	0.760
	8:35		685			0.517	20.285	0.722
	8:55		686			0.514	20.28	0.711
March 7	Cat killed. No pancreatic tissue could be found							

* Chamber not yet thoroughly ventilated.

TABLE 2

Cat 11. Effect of perfusate made with Locke's solution containing 0.5 per cent glucose

DATE	TIME	TREATMENT	NUMBER OF ANALYSIS	EXPIRED AIR		R.Q.
				Per cent CO_2	Per cent O_2	
1922	*p.m.*					
March 13	5:30		718	0.806	19.86	0.687
			719	0.705	19.99	0.682
	8:25–50	50 cc. perfusate no. 6 by femoral vein				
	8:50	Placed in respiration chamber				
	9:20		720	0.696	20.05	0.723
	9:45		721	0.726	20.07	0.782
	10:05		722	0.717	20.07	0.772
	10:30		723	0.735	20.09	0.82
	10:50	Inspired air: CO_2, 0.03 per cent; O_2, 20.87 per cent				
	11:05	Cat died of sudden hemorrhage after vomiting. No evidence of pancreatic tissue found				

Cat 11. Depancreatized March 11, 1922. Usual technique, time, one hour. On March 13, cat appeared very weak but able to walk about. Heavy reduction of Benedict's qualitative solution in urine.

Perfusate no. 6 used with cat 11 on March 13, prepared from dog's pancreas and contiguous portion of duodenum. Locke's solution containing 0.5 per cent glucose perfused through artery. No rise of pressure after first few minutes. Time of perfusion, 3 hours.

Cat 13. Depancreatized March 16, 1922. Time, 1 hour, 20 minutes. March 17, cat eats well. Perfusate no. 7 used on March 18. Prepared from pancreas only of young dog. Two hundred and fifty cubic centimeters Locke's solution used containing 1 per cent dextrose through artery. Perfusion began at 4:10 p.m.

TABLE 3

Cat 13. Negative effect of perfusate on respiratory quotient

DATE	TIME	TREATMENT	NUMBER OF ANALYSIS	EXPIRED AIR		R.Q.
				Per cent CO_2	Per cent O_2	
1922	*p.m.*					
March 18	4:30	Cat placed in chamber				
	5:25		757	0.959	19.68	0.703
	6:50		759	0.413	20.37	0.655
	7:15		760	0.676	20.04	0.703
				Average		0.687
	7:45	Cat given 40 cc. perfusate no. 7 into peritoneal cavity				
	7:50	Cat placed in chamber				
	8:25– 9:45	R.Q.'s: 0.704, 0.706, 0.679, 0.725				
	10:30–10:40	50 cc. perfusate no. 7 by femoral vein				
	11:20–12:10 a.m.	R.Q.'s: 0.657, 0.686, 0.664, 0.678				
March 23		Cat died: no pancreatic tissue could be found.				

and continued until 7:20 p.m. First 25 minutes, rate was 39 cc. per minute. Later cut down to 19 cc. per minute. Entire volume passed through 15 times. Systolic pressure 106 mm. Hg, diastolic 78 mm.

Cat 15. Depancreatized March 25, 1922. Perfusate no. 9 made with Locke's solution containing 0.5 per cent dextrose. Passed through vessels of pancreas and contiguous portion of duodenum. Rate 70 cc. per minute. Systolic pressure, 110 mm., diastolic 100. Begun March 28, 3:40 p.m.

Dog 1. Young Irish terrior, weight 8 kgm., depancreatized March 18. Pancreas carefully dissected from intestine; vessels ligated for perfusion no. 9. Perfusate given to cat 15. Dog made good recovery notwithstanding ligation and removal of pancreatico-duodenal vessels. March 20, in good condition; bowels loose, eats and drinks well.

Perfusate no. 8 made from pig's pancreas, weight 180 grams. No dextrose used. Systolic pressure 180 mm. Hg, diastolic 140, rate 40 cc. per minute. Begun 2:50 p.m., March 29.

DISCUSSION. *1. Technical.* Only those experiments which were most successful from the technical point of view are reported. Several

TABLE 4

Cat 15. Positive effect of perfusate of dog's pancreas

DATE	TIME	TREATMENT	NUMBER OF ANALYSIS	INSPIRED AIR		EXPIRED AIR		R.Q.
				Per cent CO_2	Per cent O_2	Per cent CO_2	Per cent O_2	
1922								
March 27	11:15 a.m.		839			0.594	20.16	0.723
	12:10 p.m.		840			0.544	20.19	0.709
			844	0.03	20.91			
	4:40 p.m.	40 cc. perfusate no. 9 intraperitoneally						
	4:50	Placed in chamber						
	5:15		849			0.575	20.21	0.7
	5:45		850			0.716	20.09	0.8
	6:00	40 cc. perfusate no. 9 subcutaneously						
	6:45	Placed in chamber						
March 28	7:15		851			0.695	20.06	0.755
	7:35		852			1.005	19.62	0.739
	8:00		853			0.988	19.67	0.745
	8:30		854			0.884	19.80	0.742
	8:50	40 cc. perfusate no. 9 intraperitoneally						
	10:00		856			0.887	19.825	0.761
	10:18		857			0.955	19.73	0.752
March 30	Cat died	No pancreatic tissue could be found						

others miscarried either because the animal was made so sick by the injections that it was not a fit subject for the experiment or because the preliminary checks previous to administration of the extract did not prove the animal to be completely diabetic. This is not to say that blood sugar tests or the G:N ratio in the urine might not have proven

the animal to be diabetic. Since the criterion of a distinct elevation of the respiratory quotient was being used exclusively it was necessary to start off with such a quotient as experience with diabetic dogs and human diabetics have proved to be indicative of total inability to oxidize sugar. One difficulty lay in the method of sampling the chamber air and many variations of the method described above were used. The method described is essentially that employed by Krogh and Lindhard (11) and others who have used the Jaquet or Jaquet-Grafe apparatus.

TABLE 5

Dog 1. Negative effect of perfusate of pig's pancreas

DATE	TIME	TREATMENT	NUMBER OF ANALYSIS	EXPIRED AIR		R.Q.
				Per cent CO_2	Per cent O_2	
	p.m.					
March 29	2:45	Dog placed in chamber				
			858	0.714	19.22	0.677
			859	0.698	20.00	0.699
			860	0.564	20.18	0.625
					Average	0.683
	4:15	60 cc. perfusate no. 8 subcutaneously				
	5:00	60 cc. perfusate no. 8 subcutaneously				
	5:55		862	0.757	19.91	0.689
	7:00		863	0.686	19.99	0.686
	7:20		864	0.698	20.01	0.710
	7:55	120 cc. perfusate no. 8 subcutaneously				
	9:00		865	0.664	20.05	0.710
	9:15		866	0.644	20.03	0.660
		Inspired air	867	0.03	20.90	
March 31	Dog died					

As described here it may be relied upon to give good checks with alcohol or with the totally diabetic animal.

The analyses for chamber air showed a greater variation in the content of carbon dioxide (and consequently of oxygen) than we were prepared at the outset to expect. These were found to be related to the exact position of the animal's head with reference to the exit tube; also to the rate of ventilation and to the circumstance whether or not the animal was left for a few minutes in the chamber before ventilation was started. Two instances may be noted in table 1 where this was done

and as a consequence the first sample shows a much higher CO_2 content in the chamber air than later samples. In some of the observations a small electric fan was used for stirring and mixing the air inside the chamber: but this had only a slight effect on the variations referred to. For the sake of uniformity in the analyses and the possibility of calculating the total metabolism more reliably, it was later found to be much more satisfactory to collect the chamber air in a large gasometer for a 10- or 15-minute period than to measure it continuously by means of the meter (see following paper). For the respiratory quotient alone, however, the method described here is perfectly reliable, and, as may be seen from the tables, gives good duplicate results in spite of the variations in composition of the air.

2. Effect on respiratory quotient. A glance at the tables shows at once that the pancreatic perfusates made with a weakly alkaline solution in the manner described by Clark, may increase the capacity of the organism to oxidize sugar to a slight extent. In two of the five experiments reported there was no increase at all in the quotient, and in one of the three experiments which were positive the animal died immediately after the experiment of hemorrhage after vomiting. The higher quotients in this case were, therefore, probably correct.

All of the animals were certainly completely diabetic or nearly so, (cat 15), before administration of the perfusates. At least one perfusion of cat's pancreas proved wholly ineffective on the cat. Of the six preparations reported here only one was made from the cat's pancreas, two were made from dog's pancreas together with the contiguous portion of the duodenum, two from the dog's pancreas alone and one from a fresh pig's pancreas. The greatest effects were obtained with cats 10 and 15. The former, when tested without food on the 3rd day and after feeding glucose and sucrose on the 5th day, proved to be completely diabetic. After administration of two doses, one of 30 cc. and a second of 67 cc. perfusate of cat's pancreas the *R.Q. rose about five or six points.* Four days later after proving again to be wholly incapable of oxidizing glucose, 35 cc. perfusate of dog's pancreas and duodenum raised the R.Q. from four to six points (0.696 to 0.74 or 0.76), where it remained for about 3 hours and then declined rapidly.

Cat 15 had been depancreatized only 2 days when the first basal test was made and did not show quite the typical R.Q. for total diabetes, but the quotient could not have been any higher on the 28th when the perfusate was given, for the animal had been fed on nothing but salmon. The administration intraperitoneally of 40 cc. of per-

fusate no. 9 made from the pancreas alone of a young dog caused the R.Q. to rise within 35 minutes to 0.75 and within 1 hour and 5 minutes to 0.83. A second injection of 40 cc. given this time under the skin maintained the quotient at the level 0.74 and 0.75 for 2½ hours, and a third into the peritoneum raised it once more to 0.76 and 0.75 an hour and a half after administration.

Two possible objections may be urged against the conclusion that the pancreatic hormone caused the combustion of sugar in these two animals: one, that the rise in quotient may have been due to the liberation of CO_2 from the small amount of sodium bicarbonate contained in the perfusate. This objection is met, we believe, by the control experiments run by Murlin and Kramer (12) on normal dogs where the administration by mouth of from 2 to 5 grams sodium carbonate raised the quotient only two or three points, or not at all. Furthermore, if the bicarbonate were the cause of the rise in quotient the rise should have been apparent in all the experiments for the bicarbonate was present in all perfusates. Two, the perfusate used with cat 15 contained 0.5 per cent glucose and if the animal was not completely diabetic there may have been a residual capacity to oxidize glucose even without the aid of the pancreatic hormone contained in the perfusate. This would be a legitimate objection in the case of cat 15 if the observations stood alone, but it could not apply to cat 10 which was proved to be wholly unable to oxidize sugar, and the rise in quotient in that case was the same as in cat 15. The animal died before the experiment could be controlled further. We are certain that in these two cases there was a positive effect of the pancreatic perfusate, and that the method of extracting the active principle essential to the combustion of sugar exployed by Clark may at times withdraw a sufficient amount to restore to the diabetic animal the power to oxidize sugar to a slight extent. But the method is faulty in the essential particular that the extracting medium was not made distinctly acid in reaction.

SUMMARY

1. Perfusates of the pancreas of cat, dog and pig were made by the method of Clark, employing Ringer's or Locke's solution containing 0.2 per cent sodium bicarbonate and, at times, glucose to 0.5 or 1.0 per cent.

2. In several instances which are presented in detail there was a very perceptible increase in the respiratory quotient in animals which just previously had been wholly incapable of oxidizing glucose.

3. This method of extraction (by perfusion with a weakly alkaline fluid) is however incapable of withdrawing a sufficient amount of the anti-diabetic substance to be of any practical importance. Acid fluids as will be seen in the next paper are much more efficacious.

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EXTRACTION OF THE ANTI-DIABETIC SUBSTANCE (INSULIN) BY PERFUSION OF THE PANCREAS

II. Influence of Acid Perfusates upon the Blood Sugar, D:N Ratio and Respiratory Metabolism of Depancreatized Dogs

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Acid media for the preparation of an anti-diabetic substance were first used, so far as we are aware, by E. L. Scott (1). His object in using the acid (acetic) apparently was to inactivate any possible trace of trypsin which might have been present after extraction with alcohol. Our object was rather to facilitate the solution (extraction) of the active substance since perfusates even with alkaline solutions did not show any tryptic action. The idea of employing acid for this purpose grew out of the experience of Murlin and Kramer (2) who employed N/5 HCl for the purpose both of inactivating trypsin and facilitating solution. After this work was well under way we received the second paper of Banting and Best (3) and learned for the first time that they had used 0.2 per cent HCl in 95 per cent alcohol apparently for the same twofold purpose.

Our first successful experiment with acid perfusate was performed on June 5, 1922 when a dog's pancreas weighing 25 grams was perfused with 300 cc. of the following fluid.

NaCl—27.0 grams
KCl—1.26 grams
$CaCl_2$—0.72 gram
HCl—12 cc. sp. gr. 1.19
Water—3000 cc.

Perfusate no. 10. Pancreas perfused with fluid in manner described in previous paper for 1 hour and 12 minutes at the rate of 88 cc. per minute. The total volume passed through the pancreas 21 times. Pressure systolic, 280 mm. Hg,

diastolic 200 mm. First few drops came through dark brown in color but the solution became lighter in color so that final appearance was light yellow.

This was immediately tested for potency on dog 4, depancreatized on May 27th, with the result shown in table 1 and chart 1.

Perfusate no. 11. June 8, 1922. Pig's pancreas weighing 51 grams. Brought from slaughter house in sterile thermos jar. Connected to apparatus about 1 hour after removal from animal. Perfusion fluid same as no. 10. Volume 275 cc., rate 44 cc. per minute. Time, 62 minutes. Perfusate gradually assumed light yellow color and at end contained a light flocculent precipitate. Pancreas gradually became swollen as perfusion progressed. Injected with India ink, sectioned with razor and examined under microscope. Injection mass seen in smallest capillaries in head and body of pancreas.

Perfusate removed from incubator and preserved in sterile bottles in ice box.

In all, nine 20 to 25 cc. doses of this perfusate were administered subcutaneously to dog 4, June 8th to 12th, with results shown in table 1.

Perfusate no. 12. June 13, 1922. Simultaneous perfusion of two pig pancreases weighing 140 grams. Perfused without perfusion chamber by suspending organs over a funnel inserted into bead chamber (fig. 1 of previous paper). Organs were brought from slaughter house in sterile thermos jar. Surfaces washed several times in sterile salt solution. Much difficulty was experienced in cannulation of arteries, with the consequence that perfusion was not started for 2 hours after receiving organs.

Perfusion fluid same as no. 10 and no. 11 kept meantime in sterile volumetric flasks. Volume 300 cc., rate 110 cc. per minute. Pressure systolic 70 mm. Hg diastolic 10 mm. Two pancreases perfused in parallel for 1 hour, then separately for 15 minutes. Injection with India ink showed many vessels not reached by perfusion fluid. Perfusate became milky. Placed in sterile bottles in ice box.

Two 25 cc. doses of no. 12 were given subcutaneously to dog 4 on June 13th and 14th.

Protocol, Dog 4, small, white, long-haired male. Depancreatized May 27, 1922. Operation uncomplicated. On second day had G:N ratio of 3.61. Dog made speedy recovery with help of pancreatic extracts (see later paper). Up to June 4th was given all-told seven doses of extracts made from dog and pig's pancreas. At time perfusates were begun was in good condition, wound healing, appetite good, maintaining weight. Dog lived 30 days. No macroscopic evidence of pancreatic tissue.

Of the three perfusates just described and tested on dog 4, no. 11 proved to be the most potent as judged by the fall in blood sugar on June 8th. The urine had been rendered sugar free by administration of pancreatic extracts (see next paper) before the perfusates were used, but perfusate no. 10 had served to maintain the urine in this condition throughout June 5th and 6th and two doses of this preparation had lowered the blood sugar on June 6th from 0.190 to 0.049. The next day, June 7th, no pancreatic material was given with the consequence

TABLE 1

Effect of perfusates of dog's and pig's pancreas

Dog 4. Pancreatectomy May 27, 1922

DATE	FOOD	TREATMENT	BLOOD SUGAR		URINE				REMARKS
			Time	Amount	Time	Sugar	Nitrogen	D:N	
				per cent					
June 4 (8th day)			9:00 a.m.	0.244					
June 5		6:00 p.m. 100 cc. no. 10 s.c.*	9:45 p.m.	0.148					
June 6		10:00 a.m. 50 cc. no. 10 s.c.	9:45 a.m.	0.190	9:45 a.m.	0			Previous to this dog had been given various extracts
			5:15 p.m.	0.061	2:00 p.m.	0			
		9:40 p.m. 25 cc. no. 10 s.c.	9:30 p.m.	0.049	Evening	0			
June 7		None	9:35 a.m.	0.121	Morning	0			
June 8		1:30 p.m. 25 cc. no. 11 s.c.	1:20 p.m.	0.200	1:20 p.m.	2.35	0.95	2.47	
		5:45 p.m. 25 cc. no. 11 s.c.	5:45 p.m.	0.078	5:45 p.m.	0.50	1.20	0.42	
			10:00 p.m.	0.038					
June 9	30 gm. cracker meal				6:30 a.m.	0			
		1:00 p.m. 25 cc. no. 11 s.c.	1:00 p.m.	0.178					
		5:00 p.m. 25 cc. no. 11 s.c.	5:00 p.m.	0.135	1:30 p.m.	0			
June 10	200 gm. meat, 60 gm. cracker meal				Night	4.09	1.26	3.17	
		11:30 a.m. 25 cc. no. 11 s.c.			12:30 p.m.	8.9%			Could not get blood
		5:30 p.m. 25 cc. no. 11 s.c.			5:00 p.m.	3-5%			
June 11	Same	11:00 a.m. 20 cc. no. 11 s.c.							
June 12	100 gm. meat	11:30 a.m. 25 cc. no. 11 s.c.	11:30 a.m.	0.308	6:00 a.m.	7.4	1.464	5.06	
		4:15 p.m. 25 cc. no. 11 s.c.	4:15 p.m.	0.177					
June 13	100 gm. meat				6:30 a.m.	0.952	0.902	1.05	
		4:50 p.m. 25 cc. no. 12 s.c.	10:30 a.m.	0.258	5:45 p.m.	5.26	0.925	5.68	
June 14	100 gm. meat	10:00 a.m. 25 cc. no. 12 s.c.			6:30 a.m.	3.33	1.25	2.66	
June 15	100 gm. meat				6:30 a.m.	1.39	2.03	0.86	

* s.c. means subcutaneously.

JUNE 5 JUNE 7 JUNE 8 JUNE 9 JUNE 12 JUNE 13 JUNE 14
6 P.M. 9 A.M. 1 P.M. 5 P.M. 6 A.M. 4 P.M. 10 A.M.

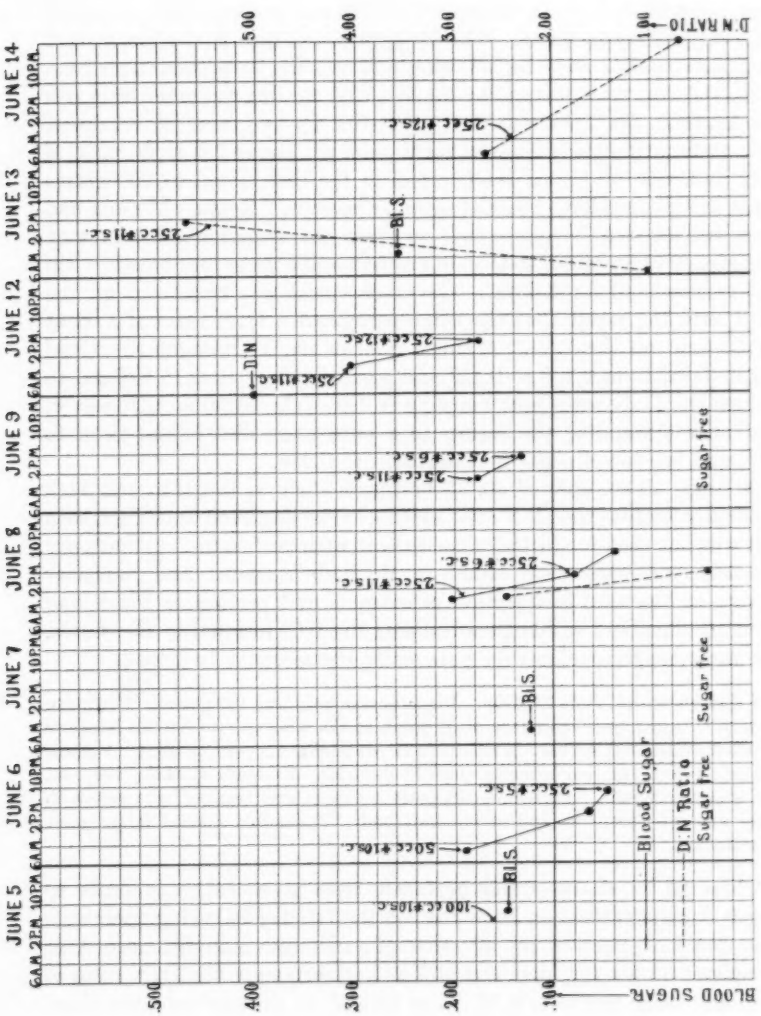

Fig. 1. Chart showing the effects on blood sugar and D:N ratio of dog no. 4 of injection of perfusates of pancreas subcutaneously. The perfusates are described in the text.

that the blood sugar rose to 0.200 at 1:20 p.m. on June 8th. One 25 cc. dose brought it down in 4½ hours to 0.078 and a second dose in a like interval of time to 0.038. The D:N ratio fell during the first period from 2.47 to 0.42 and the following morning the urine was sugar free.

On the 9th, notwithstanding the fact that the dog was given 30 grams crackermeal, the urine was kept sugar-free by two more 25 cc. doses. The night urine the following night however showed a D:N ratio of 3.17 and with 60 grams crackermeal in the diet added to 200 grams meat there was a large amount of sugar present. Because the dog had been subjected to venapuncture so often the available veins became completely obliterated, so that it was impossible to secure blood (without venesection) for 2 days. The crackermeal was continued on the 11th with the result that the urine contained a D:N ratio of 5.06 at 11:30 a.m. on the 12th. A single dose of no. 11 brought the blood sugar down from 0.308 to 0.177 and a second dose at 4:15 sufficed to reduce the D:N ratio next morning to 1.05. At 10:30 a.m., however, and notwithstanding the return to a pure meat diet it had risen to 5.68 and the blood sugar had returned to 0.258.

A single 25 cc. dose of no. 12 perfusate each on the 13th and 14th gradually lowered the D:N ratio to 0.86. The dog lived until June 26th but was not used for further experiments with perfusates. At autopsy extensive necroses were found in the subcutaneous and muscular tissues at the sites of injection of extracts and perfusates which had been administered without neutralizing the HCl residual in the fluids. No macroscopic evidence of pancreatic tissue could be found.

Perfusate no. 18, June 23, 1922. Pig pancreas weighing 180 grams. Perfusion started within 1 hour after receipt of organ at the laboratory. Sterile Ringer's solution similar to that used in nos. 10 to 12 containing 0.2 per cent HCl. Volume 410 cc., rate 57 cc. per minute, time 75 minutes. Pressure systolic, 130 mm. Hg, diastolic 96 mm. Perfusate distinctly milky at first became light yellow in color and opalescent at end of one-half hour. India ink showed fairly good penetration.

Three 25 cc. doses of this perfusate were given subcutaneously to dog 9 depancreatized on June 21st, on the 7th day following operation. The dog had received an injection of pancreatic material (record incomplete) on the day previous and as a consequence the D:N ratio in the night urine was only 0.76. The first 25 cc. dose reduced the blood sugar percentage from 0.280 to 0.156 but the second injection at 2:15 apparently was not so effective; for the blood sugar rose to 0.225 at 4:45 p.m. and the D:N ratio at 5:50 was 2.11. However, the night

TABLE 2

Effect of perfusate of pig's pancreas

Dog 9. Pancreatectomy June 21, 1922

DATE	WEIGHT	FOOD	TREATMENT		BLOOD SUGAR		URINE				REMARKS
			Time	Extract	Time	Amount	Time	Sugar	Nitrogen	D:N	
						per cent					
June 27	13.5	20 gm. beef heart	Record of injection incomplete								Condition good; eats well
June 28	13.4	450 gm. beef heart	9:50 a.m.	25 cc. no. 18 perf. s.c. faintly acid	9:45 a.m.	0.280	Night	0.416	0.595	0.76	Condition fair
			2:15 p.m.	Same	1:45 p.m.	0.156					
			6:00 p.m.	Same	4:45 p.m.	0.225	5:50 p.m.	2.71	1.28	2.11	
June 29	12.8	500 gm. beef heart	10:00 a.m.				Night	0.00			No toxic effects of perfusates
			10:15 a.m.	95 cc. no. 18 perf. by stomach tube			9:10 a.m.	0.376	0.415	0.91	
					9:40 a.m.	0.251					
					11:30 a.m.	0.300					
					12:30 p.m.	0.286					
					2:30 p.m.	0.307	2:30 p.m.	Large amount sugar			
June 30							Night	Large amount sugar			
July 1	Dog died of effects of extracts given June 29th, 6:30 p.m., and June 30th										

TABLE 3

Effect of perfusate of dog's pancreas

Dog 25. Pancreatectomy August 12, 1922

DATE	WEIGHT	FOOD	TREATMENT	BLOOD SUGAR		URINE				RESPIRATORY METABOLISM				REMARKS
				Time	Amount	Time	Sugar	Nitrogen	D:N	Time	CO_2	O_2	R.Q.	
1922					*per cent*						*cc. per min.*			
Aug. 14	5.45	Ground beef. 12:00 m. 20 gm. glucose											0.74	
										2:00 p.m.			0.76	
													0.74	
										7:30 p.m.			0.69	
													0.72	
Aug. 15		Ground beef		8:30 a.m.	0.239									
			1:00 p.m. 25 cc. no. 21 perf. s.c. neutral			2:30 p.m.	2.33	1.360	1.71					Absorption of perfusate very slow
				3:00 p.m.	0.285									
				4:50 p.m.	0.272									
				6:00 p.m.	0.230									
										7:45 p.m.			0.74	
													0.76	
Aug. 16		150 gm. ground beef	No treatment			6:30 a.m.	3.08	3.56	0.86	9:00 a.m.	42.9	54.4	0.79	Higher R.Q.'s due to slow absorption of active principle
				9:05 a.m.	0.262								0.79	
						12:00 m.	0.82	1.15	0.74				0.79	
										2:45 p.m.	63.6	78.9	0.81	
											63.6	75.5	0.84	
				4:00 p.m.	0.292						63.6	77.9	0.82	

Aug. 17	5.24	160 gm. ground beef 12:00 m. 20 gm. glucose. Vomited. Later drank glucose solution	11:15 a.m. 50cc. no. 21 perf. s.c. neutral	11:10 a.m.	0.216	6:30 a.m.	6.17	4.96	1.24	10:10 a.m.	50.8	67.5	0.75	Dog moved about
											53.8	67.5	0.75	
				1:00 p.m.	0.313					3:26 p.m.	41.6	55.9	0.73	
				3:00 p.m.	0.367						41.6	55.0	0.74	
				5:00 p.m.	0.315					7:30 p.m.	41.1	53.6	0.77	Dog's temperature 40°C.
											40.6	50.8	0.80	
Aug. 18		80 gm. ground beef				6:30 a.m.	6.53	3.44	1.89					
				10:55 a.m.	0.248					10:00 a.m.	52.8	75.4	0.73	Dog moved in respiratory chamber
			11:00 a.m. 100 cc. no. 21 perf. s.c. neutral								54.8	74.5	0.73	
		3:18 p.m. 10 gm. glucose by stomach tube. All retained		1:00 p.m.	0.192						54.8	71.7	0.75	
				3:00 p.m.	0.147					4:30 p.m.	43.5	54.2	0.80	
											43.5	52.2	0.83	
				5:00 p.m.	0.247					8:30 p.m.	38.9	56.1	0.69	Dog quite weak
											38.9	58.6	0.67	
Aug. 22	Dog chloroformed. Autopsy revealed nothing of interest													

urine was rendered sugar-free by a third injection given at 6:00 p.m. and the D:N ratio at 9:10 a.m. on the 29th was only 0.91. The rise of D:N on the 28th in spite of two injections was probably due to the large amount of meat eaten during the day.

On the 29th, 95 cc. of perfusate no. 18 were given by stomach tube, but had no demonstrable effect on the blood sugar. The urine was tested only qualitatively.

Dog 9 died on July 1st as the result of toxic effects from pancreatic extract no. 23 given intravenously (see later paper (8) for autopsy report). The perfusate apparently had no toxic effect.

Perfusate no. 21. August 15, 1922. Prepared from a dog's pancreas placed in perfusion chamber immediately after removal from the dog. Ringer's solution containing 0.2 per cent HCl. Volume 275 cc., rate 72 cc. per minute for first 15 minutes then reduced to 36 cc. per minute for 70 minutes. Pressures very high. Entire fluid passed through blood vessels 12 times.

Dog 25 depancreatized on August 12th received subcutaneously a single 25 cc. dose of perfusate no. 21 neutralized to litmus immediately after it was prepared (i.e., at 1:00 p.m.) on August 15th. There was no demonstrable effect on the blood sugar probably owing to slow absorption. But the D:N ratio which had been 1.71 soon after injection fell until next morning at 6:30 it was only 0.86. Moreover the respiratory quotient, which at 7:30 p.m. on the 14th was practically at the diabetic level, $6\frac{3}{4}$ hours after the injection, had risen a couple of points and next morning, corresponding to the low D:N ratio, was ten points above the level of total diabetes. No treatment was given on the 16th. Nevertheless the D:N ratio continued low and the respiratory quotient rose still farther to 0.82 and better up to 2:45 p.m. *It is especially to be noted that this highest level of the respiratory quotient was not reached for more than 24 hours after injection of the perfusate.* On the 17th the dog received 50 cc. of the perfusate, freshly neutralized just before injection, at 11:15 a.m., and at 12 m. was given 20 grams glucose some of which was vomited but later was drunk again. As a consequence the blood sugar rose. The R. Q. determined before injection was 0.75. Four hours after injection it had not yet begun to show any effect, but 8 hours after it had risen to 0.80.

The D:N ratio, which in an early morning cage sample (preserved with thymol in toluene) on the 17th was 1.24, 24 hours later in a similar sample (also properly preserved) in spite of the glucose ingested was only 1.89. Nearly 20 grams therefore must have been utilized.

On the 18th a still larger dose of neutralized perfusate no. 21 was given at 11 a.m. The blood sugar this time responded promptly, falling 100 mgm. in 4 hours. At this time 10 grams glucose were given by stomach tube and all was retained. The R. Q. rose in 1½ hours from 0.73 to 0.83 but in 4 hours more had fallen abruptly to the diabetic level.

In the hope of demonstrating a dynamic action of the sugar the respiratory metabolism was expressed in volumes of O_2 and CO_2 per minute. But the dog was too restless in the preliminary period to permit of a good basal determination. In fact both on the 17th and the 18th the absorption of oxygen was progressively less after administration than before. This only reflects muscular quiescence and may possibly be interpreted as a soothing effect of the perfusate rather than a real diminution of the hypermetabolism of total diabetes (4).

The dog was chloroformed on August 22nd but the autopsy revealed neither any necroses resulting from the injections nor any residual pancreatic tissue.

Perfusate no. 22. August 19, 1922 Dog's pancreas placed in perfusion chamber as soon as it could be removed aseptically from the animal. Ringer's solution containing 0.2 per cent HCl. Volume 275 cc., rate 32 cc. per minute, time 2 hours. Pancreas became very much swollen, a sharp line of demarcation separating the perfused from the non-perfused portion. Fluid stained yellowish brown. India ink showed excellent penetration. Perfusate preserved unneutralized in ice box.

Dog 26 depancreatized on August 8th, received on the 22nd two large doses of unconcentrated perfusate no. 22 neutralized just before injection. Previous to this date the animal had been treated with various pancreatic extracts and had been symptom-free much of the time. The low D:N ratio and R. Q. of 0.72 in the morning are residual effects of this treatment. Three feedings of glucose amounting to 35 grams all told were given during the day. Nevertheless the blood sugar fell from 0.247 just before first injection to 0.149, 2½ hours after the last feeding of sugar and the D:N ratio was held at about the level it had started with, and the night urine was sugar free.

The respiratory quotient beginning at 0.72, 3 hours before treatment rose to 0.85 in 5 hours but at the 8th hour had subsided several points.

Extract of pancreas instead of perfusate was given on the 23rd, and on the 24th no treatment. On the 25th 80 cc. of perfusate no. 22 were administered as usual and 35 grams glucose were put into the stomach

TABLE 4

Effect of perfusate of dog's pancreas

Dog 26. Pancreatectomy August 8, 1922

DATE	WEIGHT	FOOD	TREATMENT	BLOOD SUGAR		URINE				RESPIRATORY METABOLISM				REMARKS
				Time	Amount	Time	G	N	D:N	Time	CO_2	O_2	R.Q.	
1922	*kgm.*				*per cent*						*cc. per min.*			
Aug. 22	9.10			9:40 a.m.	0.260	9:30 a.m.	0.617	0.57	1.08					
										9:55 a.m.	98.5	137.4	0.72	Previous to this
											99.6	135.3	0.74	date dog had
		12:36 p.m. 10 gm. glucose in 100 cc. H_2O. 15 gm. by stomach tube. All retained. 2:10 p.m. 20 gm. glucose by stomach tube. All retained	12:35 p.m. 100 cc. perf. no. 22 s.c.	12:25 p.m.	0.247	12:30 p.m.	1.58	0.79	2.00		99.6	141.6	0.70	been treated with various extracts (see paper)
			2:00 p.m. 50 cc. perf. no. 22 s.c.											
				5:30 p.m.	0.149					5:25 p.m.	70.2	83.1	0.85	
											70.2	83.1	0.85	
						6:30 p.m.	2.52	1.27	1.98		69.23	83.1	0.83	
										8:20 p.m.	66.33	90.3	0.74	
											66.33	83.5	0.79	
Aug. 23	8.56	160 gm. ground beef	Given extract (see dog 26 later paper)			9:30 a.m.	0.0							
Aug. 24	8.65	250 gm. ground beef	No treatment			Night	4.90	2.88	1.70					

Aug. 25	8.65					Night	10.33	3.78	2.73					
				11:20 a.m.	0.312	11:15 a.m.	2.04	1.72	1.18					
		12:05 p.m. 25 gm. dextrose by stomach tube. Drank also 10 gm. dextrose	12:00 m. 80 cc. no. 22 perf. s.c.											
		4:20 p.m. 250 gm. ground beef		4:20 p.m.	0.278									
Aug. 26	8.65					Night	6.52	0.97	6.68					Abdominal wound almost completely healed
		12:00 m. 260 gm. ground beef. Ate all	Extract (see dog 26 paper on aqueous extracts)	11:15 a.m.	0.339	11:10 a.m.	2.03	2.02	1.00					
Aug. 27	8.70	8:00 a.m. 250 gm. ground beef. Ate all	Extract											
Aug. 28	8.30					10:00 a.m.	2.33	1.00	2.33	11:00 a.m.	42.8	54.7	0.78	
		12:20 p.m. 40 gm. glucose by stomach tube. All retained	12:05 p.m. 75 cc. perf. no. 22 s.c.	12:00 m.	0.336						42.8	53.9	0.79	
						5:00 p.m.	1.60	0.93	1.61	6:15 p.m.	44.4	56.3	0.79	
											44.4	56.3	0.79	
Aug. 29	8.30					4:00 p.m.	2.52	0.87	2.90					

immediately afterward. Ten grams more were taken voluntarily and all of it was retained. The single injection served to hold down the blood sugar.

A very similar experiment was repeated on the 28th after two intervening days when extract was used. This time the effect on blood sugar was not recorded, but the D:N ratio was restrained from rising notwithstanding the large ingestion of sugar. For some reason the R. Q. up to 5 hours showed no increase, although good duplicate analyses were obtained. Probably, as with dog 25, the delay was due to slow absorption of the extract from the subcutaneous spaces; for all the salts were still present in the perfusate.

METHOD FOR RESPIRATORY METABOLISM. A slightly different method of obtaining the respiratory exchange was employed in this series. The same respiratory chamber was used and the cage air was analyzed by the Haldane method just as in the previous series. But the entire air for a given period was driven by a small blower directly from the chamber into a gasometer where it was measured after which the volume was reduced to standard conditions. This method has the advantage over the original Jaquet method of permitting many duplicate analyses, if need be. The method of sampling from the gasometer is also probably somewhat more accurate. At all events good checks with the alcohol flame were obtained very readily and with completely diabetic animals the quotients were seldom above 0.69.

The assemblage of apparatus is shown in fig. 2. Samples of ingoing air could be taken from the side tube S. I. Differences between the analysis of ingoing and that of chamber air give the respiratory exchange in percentages. The R. Q. can be computed directly from these differences; but can be obtained also by dividing the total carbon dioxide exhaled by the total oxygen inhaled. The latter method was employed in this series and the total respiratory exchange in cubic centimeters of each gas per minute is given in the tables.

DISCUSSION. In none of the experiments recorded in this paper were the perfusates concentrated. In fact they were used without further treatment save that of neutralizing the free acid which had already been considerably reduced by absorption by the pancreatic tissue in the course of perfusion. Neutralization usually threw down a copious precipitate of acid metaproteins. Further tests after filtration showed a relatively small amount of proteins present, compared with similar stages in the preparation of extracts. There was also very little fat present so that it was deemed unnecessary to employ

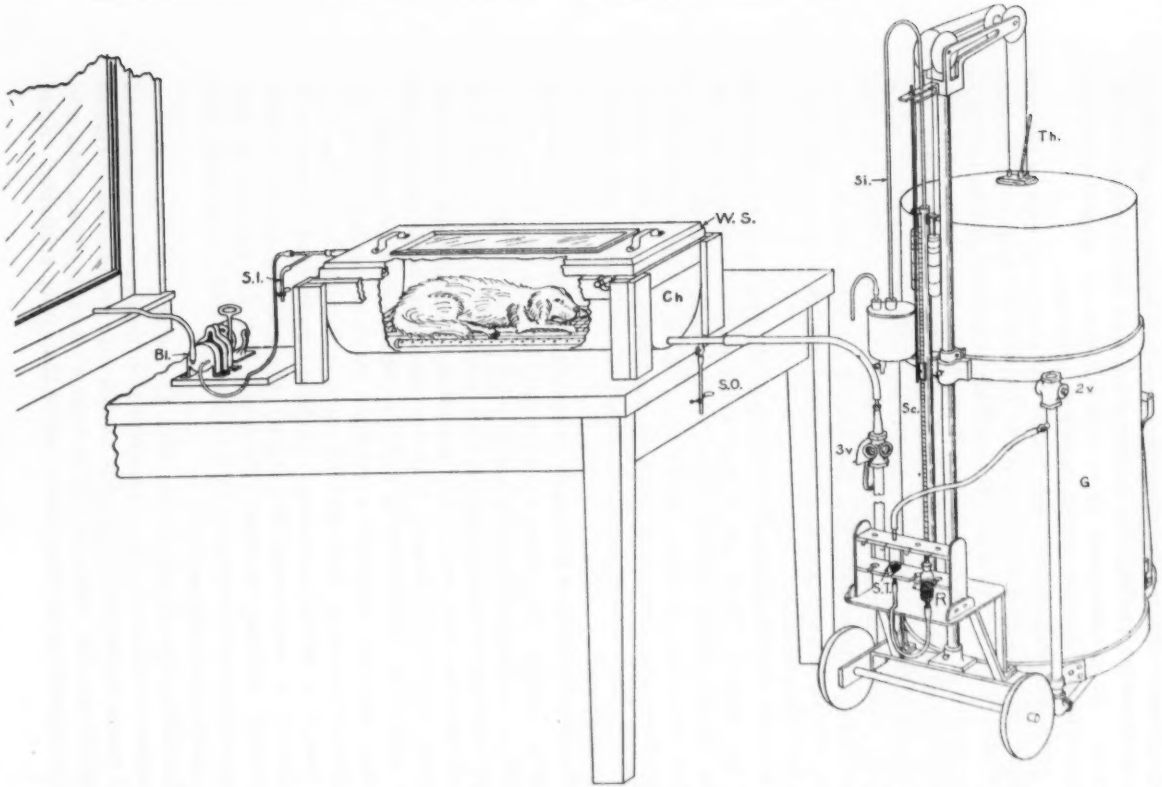

Fig. 2. Respiration apparatus combining features of the Jaquet method with some features of the Tissot method. *Bl*, small blower such as used by nose and throat specialists; *S.I.*, side tube from which samples of the ingoing air are taken; *Ch*, animal chamber; *G*, gasometer after Boothby; *Si*, siphon; *Sc*, scale for reading level of gasometer bell; *Th*, thermometer; *3v*, 3-way valve at inlet of gasometer; *2v*, 2-way valve at outlet; *S.T.*, sampling tube for taking sample over mercury for analysis.

fat solvents. The perfusion method therefore offers some advantages over extraction methods for the preparation of the anti-diabetic substance. In a subsequent paper the influence of concentrated perfusates on the diabetic symptoms will be presented.

After some experience it was found that the use of acid in the perfusion fluid obviated the necessity of the extreme aseptic precautions followed in the earlier series, the acid alone being sufficient to inhibit the growth of bacteria. Likewise it was found that the use of oxygen could be omitted since the pancreatic tissue was apparently killed after perfusion of the acid fluid for a short time. Nevertheless all the experiments reported in this paper were carried out with oxygen and at incubation temperature. The last named condition served at least to keep the tissues soft and more permeable than they would have been at room temperature.

Why do acid fluids succeed better than alkaline fluids for extraction of the insulin? There are at least three possible explanations: one, that insulin present perhaps in a zymogen stage is more soluble in dilute acids than in dilute alkali: two, that it is better preserved in acid fluids and three, that the acid inactivates trypsin and thereby prevents destruction of insulin by digestion, as Banting and Best (5) have supposed.

The islet cells have been fully described cytologically by Lane (6) and by Bensley (7). There are according to Lane two kinds of cells which he designates as A cells and B cells. The two differ slightly in their staining properties which in turn depend upon the nature of the small granules with which they are filled. The granules are slowly soluble, according to Bensley, in isotonic sodium chloride solutions and are stained by neutral red and Janus green. It is perhaps fair to assume that they represent a zymogen stage of insulin. If so, the staining reactions in fresh material indicate that insulin in this stage at least is a neutral substance. In fixed preparations (chromic-acid sublimate or acetic-osmic-bichromate) the granules exhibit marked acidophilic staining reactions (Bensley). But the reaction in fixed material does not throw much light on the nature of the material under physiological conditions.

In a later paper it will be seen that the potency of insulin may be preserved in acid media for at least four months whereas it is destroyed in a much shorter time in alkaline media. We are inclined to believe therefore that the better success in extraction by perfusion with acid media depends upon preservation rather than upon solubility. Since

we have not found any evidence of trypsin even in neutral or faintly alkaline perfusates we may safely infer that trypsinogen is not washed out by perfusion.

SUMMARY

1. Insulin is extracted by perfusion of the pancreas with acidulated Ringer's solution more readily than with the same fluid made slightly alkaline.

2. Sufficient concentration of the anti-diabetic substance is obtained in this way to show, without further treatment of the material than mere neutralization (to litmus), a precipitate fall in both the blood sugar and D:N ratio of depancreatized dogs, following a single injection of 25 to 50 cc. subcutaneously.

3. Following a liberal feeding of glucose and a single injection of perfusate the respiratory quotient has been raised several times from the diabetic level to 0.81 to 0.85.

4. A combination of the Jaquet and Tissot methods was used for determination of the respiratory exchange.

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THE RELATIVE STIMULATING EFFICIENCY OF CONTINUOUS AND INTERMITTENT LIGHT IN THE TACHINA FLY, ARCHYTAS ATERRIMA

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It was shown previously (1) that the stimulating effect of intermittent light in Vanessa antiopa depends upon flash-frequency and that it may be greater, equal to, or less than that of continuous light of equal illumination. Since this butterfly is apparently the only organism, apart from man, in which intermittent light has been described as having at certain flash-frequencies greater stimulating efficiency than continuous light, it is desirable that the effect of intermittent light upon other organisms be investigated.

The tachina fly, Archytas aterrima, was chosen for this investigation because it is usually highly positive to light and because it could be secured in large numbers.

Before continuing the discussion of these experiments, I wish to express my gratitude to Prof. S. O. Mast whose continuous aid has made this work possible. It is a pleasure also to acknowledge my indebtedness to Dr. J. M. Aldrich for kindly identifying the flies for me and to Prof. W. H. Keeble for the unselfish loan of apparatus.

METHODS. The tachina flies used were found feeding on various species of Polygonum and Dodder on a river bank. As soon as they were caught their wings were clipped. They were kept in darkness until the time of the tests which usually occurred within 3 or 4 hours. The attempt was made to test the insects in as nearly a normal state as possible, i.e., to avoid excessive mechanical stimulation and unfavorable laboratory conditions. These precautions are necessary since the tachina flies, which are normally highly positive to light, become negative or indifferent if they are stimulated by being violently shaken.

The observations were all made on a table in a dark room in a field of light composed of two small horizontal beams, produced by two 100 watt stereopticon lamps so situated that the beams crossed at

right angles. The lamps were mounted in front of a small opening in a light-proof box that was painted dead black inside. By means of screens the light from the lamps was so cut down as to produce sharply defined beams of the size desired. These beams were the only light in the room, and this was in large part absorbed by means of black cloth which covered the walls of the room. There was consequently very little light in the room aside from that in the beams. In most of the experiments the light in one of the beams was intermittent. The intermittent light was produced by interposing in the beam a rotating sectored disk connected with a small motor which was run on storage batteries. The rate of rotation could be varied as desired by means of a rheostat in the circuit. The relative duration of the flashes and the dark periods of the intermittent light used was constant, the former being one-third as long as the latter. Different disks were however used with the following numbers of apertures, 1, 2 and 8. By the use of disks with numerous apertures intermittent light of high flash-frequency was produced with a minimum of vibration caused by the movement of the motor. The vibration not eliminated was slight and did not affect the results as was shown by tests.

Not only was the behavior of the animals observed closely by the investigator but the flies themselves were forced to make permanent records of their own behavior. This was done by allowing them to walk on sheets of paper (15 × 20 cm.) upon which very fine charcoal dust had been sprinkled with a pepper shaker. After an insect had moved across a sheet so prepared a pencil was drawn along the trail left by the organism. After removal of the charcoal the sheet bore a permanent record of the reactions of the animal. Upon the sheets bearing the tracings of the insects were also marked the limits and direction of the beams of light.

The light produced by the lamps used in the experiments was measured by means of a Sharp-Millar photometer.

Each insect was given five trials. In beginning each trial the flies faced directly the source of continuous light and started from a point in the beam of continuous light and about 5 cm. from the edge of the other beam. In analyzing the tracings made a line was drawn bisecting the angle made by the rays of light at the center of each beam. The angle that each tracing made with this line was then measured. If, for example, the tracing was at an angle of 10 degrees with the line bisecting the angle between the beams it was marked "plus" or "minus" 10 degrees, depending upon whether the fly deflected toward the left

or the right. The intermittent light was always to the left. Deflection toward it was consequently always "plus" (fig. 1).

OBSERVATIONS. Preliminary tests showed that when Archytas is exposed to continuous light from two sources, the rays of which cross at right angles, it moves toward a point between the sources, the location of this point depending upon the relation between the illumination received from the two sources. Other tests showed that Archytas reacts to intermittent light of a flash-frequency of 160 per second as it does to continuous light of equal illumination, showing that the effect of intermittent light of this flash-frequency is the same as that of continuous light. In the following experiments intermittent light of a flash-frequency of 160 per second was used in place of continuous light. Since the effect of this is the same as that of continuous light it will

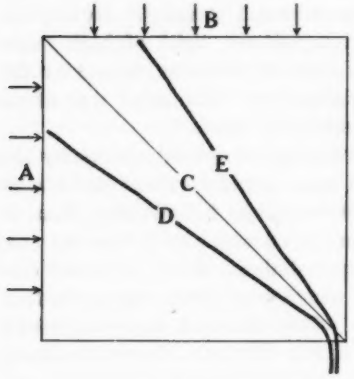

Fig. 1

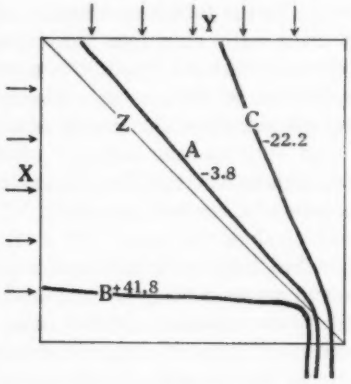

Fig. 2

Fig. 1. Diagram to show methods used in recording the reactions of Archytas in a field of light composed of two horizontal beams crossing at right angles. Arrows, direction of rays of light; *A*, intermittent light; *B*, continuous light; *C*, line bisecting the angle made by the two beams; *D*, path of fly making an angle of +10 degrees; *E*, path of fly making an angle of −10 degrees.

Fig. 2. Diagram to show paths made by a tachina fly in a field of light consisting of intermittent and continuous light of equal illumination, 35 meter-candles. *X*, intermittent light; *Y*, continuous light; *Z*, line bisecting angle made by the two beams; *A*, representation of the average path made in 5 trials when the flash-frequency of the intermittent light was 160 per second; *B*, average path in 5 trials in light of 15 per second; *C*, average path in 5 trials in light of 2 per second; figures, angles made in above trials.

be referred to in the following pages as continuous light. By this method the light sources could be kept stationary, thus decreasing the chance of error in reckoning the illumination in the beams, which a constant shifting of the light sources would have involved if continuous light had been used.

The light produced by the lamps was measured and then the lamps were so placed that the illumination from the two sources was equal (35 m.c.) in the center of the field of light common to the two beams, continuous and intermittent, which crossed at right angles. The speed of the motor was regulated so as to give intermittent light of a flash-frequency of 160 per second. In each experiment a fly was placed in the beam of continuous light, facing this source, and about 5 cm. from the edge of the beam of intermittent light. As soon as it had walked across the field of light a small fine meshed wire cone was placed over the insect. After the animal had crawled up in this chamber the cone was picked up and laid on its side so that the insect in moving toward the light began the second trial from about the same point and again facing the source of continuous light. In a similar manner three other trials were given. The cone containing the fly was then placed upon a nearby table, the flash-frequency of the intermittent light decreased to 50 per second, and again 5 trials were given as above. Similarly tests were given in intermittent light of the following flash-frequencies: 40, 30, 20, 15, 10, 5 and 2 per second. Each of 10 flies was given all of the tests described above.

While there was considerable variation in the successive trials of individuals and in the average trials of different insects, the results were striking and unquestionable. These may be illustrated by a detailed description of a typical experiment.

In this experiment the fly was first given five trials in two beams of equal illumination, one of them being of continuous and the other of intermittent light of a flash-frequency of 160 per second. The organism moved very nearly along a line bisecting the angle between the beams of light, making an average angle of -3.8 degrees. The insect was next given five tests in each of the following flash-frequencies: 50, 40, 30, 20, 15, 10, 5 and 2 per second. It made the following average angles respectively: $+10$, $+11.2$, $+15.6$, $+35.4$, $+41.8$, $+32.4$, -11.2 and -22.2 degrees. Some of the paths made in several of these tests are shown in figure 2.

Consequently, the reactions of this insect indicate: *a*, that intermittent light of flash-frequencies of 20, 15 and 10 per second has

higher stimulating efficiency in Archytas than continuous light, i.e., a given amount of intermittent light has a greater stimulating effect than the same amount of continuous light; *b*, that the maximum stimulating efficiency of intermittent light is at a flash-frequency of 15 per second; *c*, that the stimulating efficiency of intermittent light of a flash-frequency of 2 per second is less than that of continuous light; and *d*, that the stimulating efficiency of intermittent light of 5 per second is approximately equal to that of continuous light. These

TABLE 1

Relative stimulating efficiency of intermittent light of various flash-frequencies. The numbers indicate the degree of deflection from the line bisecting the angle between two beams, one of continuous and the other of intermittent light, crossing at right angles. "Plus," deflection toward the source of intermittent light; "minus," deflection toward the source of continuous light. Each figure given is the average angle of 5 trials. Illumination in both beams equal, 35 meter-candles

DESIGNATION OF FLIES	FLASH FREQUENCY PER SECOND								
	160	50	40	30	20	15	10	5	2
2	−31.4	−20.0	−26.0	−25.8	− 5.8	− 4.6	+16.2	−30.0	−34.8
10	−31.4	− 7.4	− 2.0	−13.4	−10.0	+21.4	+ 7.6	−14.4	−10.2
9	− 1.0	− 2.0	− 9.8	− 1.8	+ 0.2	− 1.8	+13.2	−12.6	−19.8
9-a	+ 1.2	0	+14.8	+27.6	+15.4	+42.0	+31.8	+17.8	+ 0.2
3	−12.0	+ 5.0	+16.8	+27.2	− 7.6	+24.0	+ 8.4	+ 1.0	−47.0
6	+ 1.0	+ 9.8	+10.2	+11.0	+39.2	+30.0	+41.0	+16.0	− 2.4
4	−21.2	−10.8	−15.6	+15.4	− 2.2	+25.0	+ 3.4	− 0.4	−18.4
4-a	− 3.8	+10.0	+11.2	+15.6	+35.4	+41.8	+32.4	−11.2	−22.2
10-a	− 4.6	+ 2.25	− 2.0	+ 8.6	+11.2	+17.4	−10.2	− 6.0	− 5.0
8	−18.8	−34.6	+ 5.0	+18.5	+28.0	+27.8	− 2.8	−20.0	−30.0
Total average angles	−12.2	− 4.77	+ 0.26	+ 8.29	+10.38	+22.3	+14.1	− 5.98	−18.96

results are in general strongly supported by the results of all of the tests made which are presented in table 1.

The last line in table 1 indicates that light of flash-frequencies of 50, 40, 30, 20, 15 and 10 per second is more efficient than continuous light; that the maximum efficiency is at about 15 per second; and that as the flash-frequency is increased above and decreased below 15 per second the stimulating efficiency of intermittent light is lowered. These two statements are based on the facts: *a*, that the average angle of all 10 insects in light of 15 per second was +22.3 degrees,

34.5 degrees greater than that made in continuous light; and *b*, that the average angles made in the other flash-frequencies just mentioned decreased as the flash-frequency was raised above 15 per second and as it was lowered below this point. The conclusion that the maximum efficiency is probably reached at 15 per second is supported by the fact that this was the flash-frequency of maximum stimulating efficiency for 5 out of the 10 flies tested. That some of the insects however showed variation in the flash-frequency of maximum stimulating efficiency is shown by the fact that for 3 flies this point was 10 per second, for 1 fly it was 30 per second, and that for 1 insect it was either 20 or 15 per second, almost the same average angle being made in light of each flash-frequency.

Examination of this table also shows that light of a flash-frequency of 2 per second is clearly less efficient than that of continuous light, since the average angles of deflection of 8 out of the 10 flies was less in intermittent light of this flash-frequency than it was in continuous light. Moreover, the average angle of all 10 insects was 6.96 degrees less in the former than in the latter light.

Furthermore, table 1 shows that probably light of a flash-frequency of between 5 and 2 per second has the same stimulating efficiency as continuous light since, as stated above, light of 2 per second is less efficient than continuous light, and light of 5 per second is slightly more efficient than continuous light, the average angle of all 10 insects in light of 5 per second being −5.98 degrees, 6.22 degrees greater than that made in continuous light.

It is probable that light of a flash-frequency of slightly over 50 per second is equal in efficiency to that of continuous light since the average angle of all 10 insects in light of 50 per second was −4.77 degrees, being only slightly greater than that made in continuous light. Moreover, since the average angles decreased as the flash-frequency was raised from 15 to 50 per second it is probable that it would have decreased still further as the flash-frequency was raised above 50 per second.

The conclusions stated above are in harmony with those previously reached with reference to Vanessa (1) and are in general supported by the results of experiments on the aphid, Macrosiphum ambrosiae, and the drone-fly, Eristalis tenax, to be published in later papers.

Mast (2) maintains that the fact that intermittent light has at certain flash-frequencies a higher stimulating efficiency than continuous illumination indicates that the processes involved in stimulation are not continuous. He contends that there are in the nervous system

alternate sensitive and refractory periods and that stimulation occurs during the former and restitution during the latter.

SUMMARY

1. When Archytas aterrima is exposed to continuous light from two sources, the rays of which cross at right angles, it moves toward a point between the sources. The location of this point depends upon the relation between the illumination received from the two sources.

2. In an illumination of 35 meter-candles the stimulating efficiency of intermittent light in the orientation of Archytas varies with the flash-frequency. At flash-frequencies of 50, 40, 30, 20, 15 and 10 per second it is higher than that of continuous light, the maximum being at 15 per second; at a flash-frequency of 2 per second it is lower than that of continuous light; and at flash-frequencies of 5, 60 and 160 per second it is approximately equal to that of continuous light.

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THE RELATIONS BETWEEN GASTRIC AND DUODENAL PERISTALSIS

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It is well known to physiologists and radiographers that the gastric waves appear to stop at the pylorus. Sometimes it seems as if the occasional contraction which succeeds in pushing some food out of the stomach runs on down the duodenum, but a more careful study, especially of plates taken in rapid succession, will show that the wave in the duodenum starts generally from the distal end of the "cap," and that it departs a few seconds before the corresponding gastric wave reaches the pylorus. In most cases an observer watching the human stomach with the Roentgen ray will be satisfied that the waves are dying out or fading away in the antrum, where they seem to be blocked by contractions of the pyloric ring.

Alvarez was first led to question the completeness of this block in 1913, when, with the help of a simple little enterograph (1), he was able to get simultaneous records from several parts of the digestive tract at one time. A number of these records showed what appeared to be tonus waves in the upper part of the duodenum, waves which were almost synchronous with those in the pars pylorica of the stomach. This interesting finding was not published at the time because it was feared that the rhythmic changes in the duodenal record might, in spite of all precautions, be artefacts due to the stomach's pulling or pushing on one arm of the enterograph. Some note was made later, however, of an observation about which there was no doubt, and that was that the peristaltic rushes in the bowel almost always start from one of these apparent tone changes in the duodenum (2).

Attention was again directed to these phenomena in 1921, when the writers began to study electroenterograms secured simultaneously from the stomach and duodenum. As will be seen from figure 1 *A* there could be no doubt about the presence of electrical disturbances in the

duodenum corresponding to those in the stomach. The next question was: Were these electrical currents generated by contractions of the duodenal muscle or were they developed in the stomach and trans-

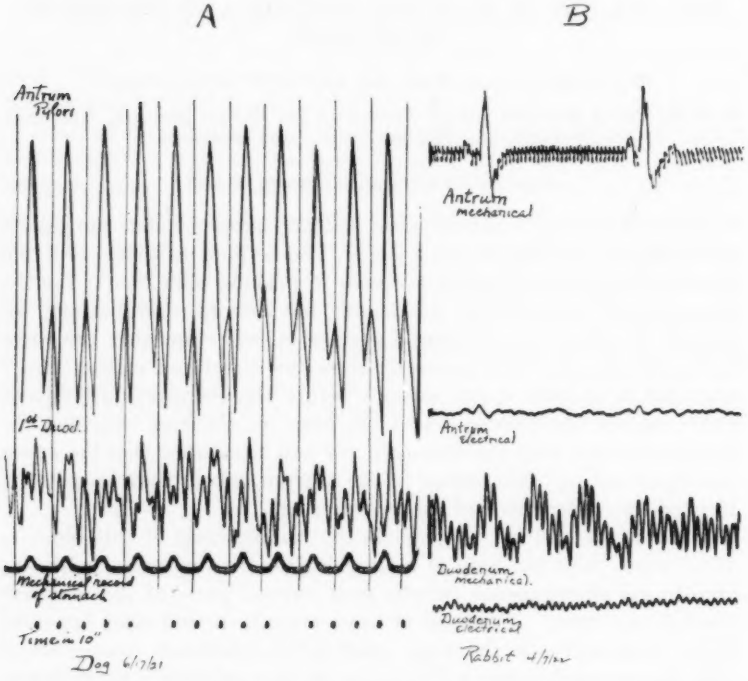

Fig. 1, *A*. Shows: From above downward, electrogram from the antrum pylori; electrogram from the first portion of the duodenum; mechanogram from the antrum pylori; time record representing ten second intervals. The animal was a dog with the abdomen opened and the bowel under moist gauze. The vertical lines have been drawn to show corresponding points in the records. In all of the illustrations an upward excursion of the light spot in the electrogram indicates negativity of the orad electrode; in the mechanogram it indicates contraction of the muscle. Note the "tonus" waves in the duodenum practically synchronous with the arrival of the gastric waves in the antrum.

Fig. 1, *B*. Shows: From above downward mechanogram from the antrum of a rabbit's stomach; electrogram from the same region; mechanogram from the first portion of the duodenum; electrogram from that region. Note big duodenal tonus waves without corresponding waves in the stomach. The small waves in the gastric record are respiratory in origin.

mitted to the duodenum along moist tissues serving as conductors? Such questions in heart work are generally settled by an examination of the records because, naturally, when the conduction is electrical the deflections in the records made from any two regions are synchronous, but when the conduction is maintained as a wave of muscular contraction, a measurable interval can be found. The problem is more difficult in the digestive tract because the movements are so sluggish, and it is so hard to determine points of take-off on the records. Some of the electroenterograms showed a definite interval between the deflections in stomach and duodenum while others did not. (See figs. 2*C* and *B*.)

In order to settle the doubts that remained, new apparatus was devised and trustworthy mechanical records were obtained from the pyloric region in many animals. These records showed clearly that the tone of the duodenum often rises and falls with the activity of the lower end of the stomach. Just as we were about to send off our report on the subject there appeared an article by Wheelon and Thomas (3) which described many of the phenomena which we had been studying. They used an ingenious set of metal tambours which were anchored in the pyloric canals of dogs in such a way as to record simultaneously the movements of the antrum, the sphincter, and the first portion of the duodenum. Their tracings leave no doubt about the existence of a correlation between the activities of the pyloric antrum and the upper duodenum. On searching the literature they found also a brief note published in 1910 by Joseph and Meltzer (4) to the effect that "during each contraction of the pyloric part of the stomach the duodenum stops its rhythmic activity and loses its tone." It is probable that if Joseph and Meltzer had placed their recording balloons higher up in the duodenum they would have found, as we do, that the duodenal rise in tone is nearly synchronous with the arrival of a gastric wave at the pylorus.

As there were a few differences between our observations and conclusions and those of Wheelon and Thomas, we have spent several months more on experimental work and have rewritten our paper. We believe it has been a good thing to go over the problem again with different species of animals and a different technic. Whereas they used balloons anchored in the pyloric canal we have used delicate recorders fastened to the outside of the stomach and bowel.

TECHNIC. We used rabbits, dogs and cats anesthetized with urethane or ether. The dorsolumbar cord was pithed from below and the usual midline incision was made. As it was essential to get a good

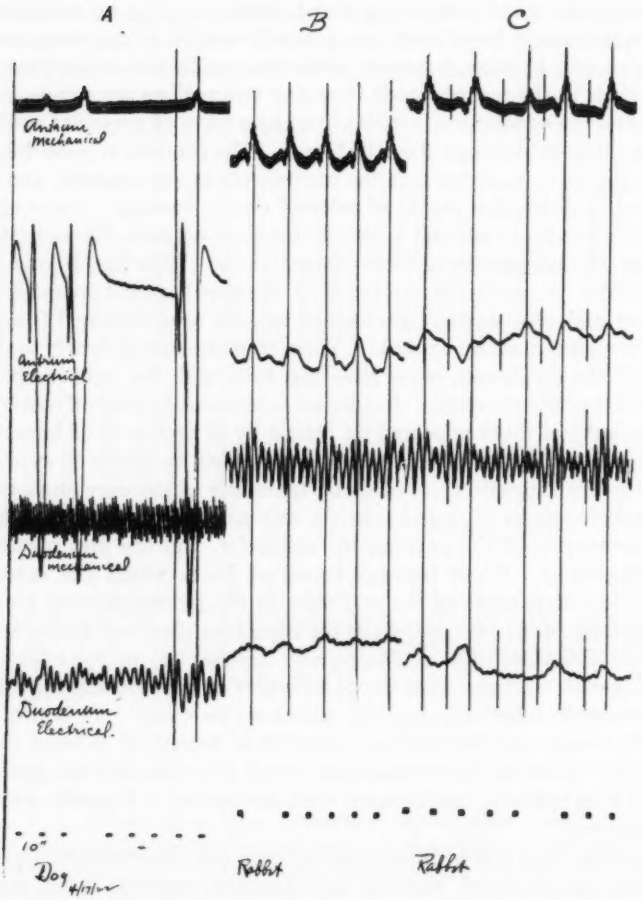

Fig. 2, *A*, *B*, *C*. Show: From above downward mechanogram from the antrum pylori; electrogram from that region; mechanogram from the first duodenum; electrogram from the same region. *A* is from a dog; *B* and *C* are from a rabbit. The frequent waves are respiratory in origin. *A* is to show the lack of current spread at times. *B* shows that the tonus wave in the duodenum may be synchronous with the antral wave; and *C* shows that a little later in the same animal the duodenal wave appeared slightly ahead of the gastric one.

exposure of the pyloric region, the abdominal wall just below the right costal margin was seized firmly in the jaws of a long, powerful Pean clamp which controlled hemorrhage and held the different layers together while a cut was made from the tip of the sternum to the region of the right kidney. The edges of this cut were then whipped over with thread, and the animal was put into a bath of Locke's solution at 38°C.

The mechanical records shown in this article were secured with the help of the enterograph which is pictured in figure 3. The little wire serrefine fixed at the lower end of a quarter inch brass rod serves to immobilize a point on the stomach or bowel. Fortunately, the grip of such a serrefine on the peritoneal coat and underlying muscle is so delicate that it does not seem to affect peristalsis in any way. Another point, perhaps 2 cm. distant from the first, is picked up by another serrefine which is fastened to a thread which runs under the little pulley placed at the end of the horizontal bar, and up to the usual light heart lever. The magnification is 4.5 times. By using a number of such recorders at one time we can easily obtain simultaneous records from several parts of the stomach and bowel. The enterographs are much better than balloons because *1*, they can be attached with almost no trauma; *2*, they do not obstruct the passage of the intestinal contents; *3*, the bowel does not struggle to get rid of them as it does with balloons; *4*, they do not slip about; *5*, they can be made to record either the circular or the longitudinal contractions; and *6*, they give a record of the actual amount of shortening or lengthening of the muscles situated between the two tiny clamps.

As the pyloric region of the stomach is directed backwards toward the hilum of the liver in most of the animals, the antral records can best be obtained by fixing the pylorus with a serrefine on the end of a rod and then fastening another serrefine to the gastric serosa some 2 cm. above. The thread from this serrefine can run directly to the end of the recording lever. That means that the sharp rise in the antral records marks the arrival of a gastric wave not at the pylorus, but at the upper serrefine 2 cm. away. This must be kept in mind in studying the time relations between the gastric and the duodenal waves. In figure 4 and in figure 6 *A* the gastric tracing was secured with the help of a small enterograph which was fastened to the peritoneal coat so near to the pylorus that the little humps on the record correspond very closely with the arrival of the visible waves at the sphincter.

Figure 4 shows how marked an effect the arrival of the gastric waves at the pylorus can have on the tone and activity of the duodenum. As one would expect, these effects are best seen in the first few centimeters of the bowel, but they can sometimes be recognized in records

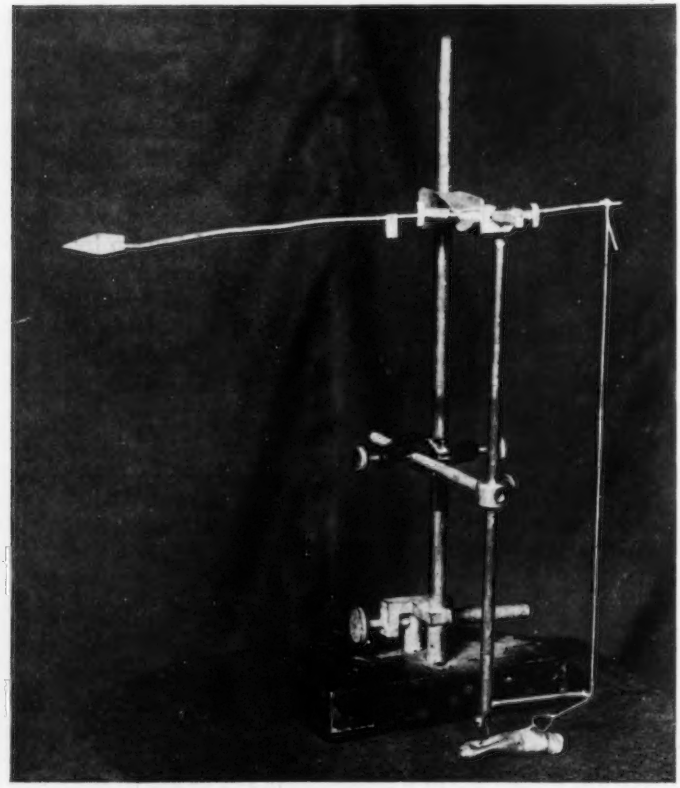

Fig. 3. Enterograph described in the text.

made in the lower duodenum, and sometimes even in the jejunum. (See fig. 5.) The study of the time relations between the gastric waves and the "tone rises" in the duodenum is complicated somewhat by the fact that the large rises in both stomach and bowel are sometimes preceded by short drops which may be due either to inhibition or to a

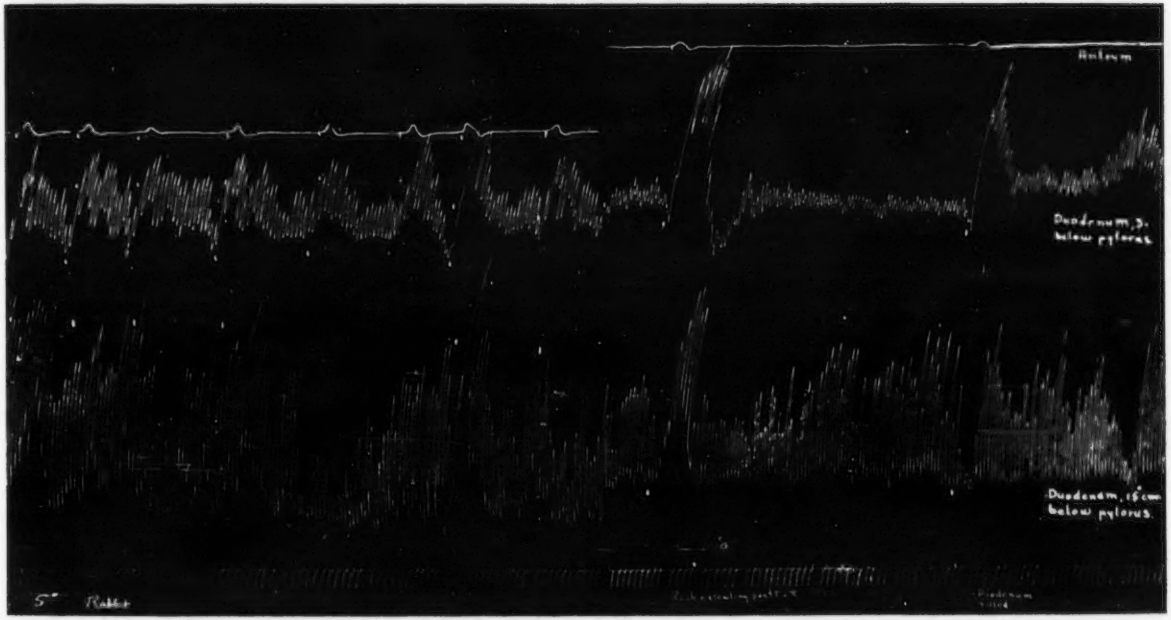

Fig. 4. Tracings from the pyloric antrum and two points on the upper duodenum of a rabbit. The marks show corresponding points on the records. The beginning of the rise in the gastric "hump" marks the arrival of a wave at the pylorus. Time record indicates 5-second intervals with a break every minute.

stretching of the muscle by fibers contracting just above. At other times the gastric record is dicrotic in appearance, and it is hard to say just when the main wave arrived at the pylorus. Often, however, as in the upper left hand corner of figure 5, the contractions are recorded so simply and so sharply that we can say that the duodenal tonus wave, even 23 cm. from the pylorus, appeared some 4 seconds before the corresponding gastric wave approached the pylorus. A similar precedence of the duodenal wave will be found in figure 2 *C*. In figure 4 it will be seen that the duodenal rise comes about the same time or shortly after the arrival of the gastric wave at the pylorus, but there is a preliminary relaxation which appears several seconds before the antral wave begins. In other records the duodenal contraction follows the gastric wave after a short interval, much as it does in the tracings published by Wheelon and Thomas. In still other records the gastric waves are so regular that one cannot tell whether a given duodenal rise corresponds to the wave just before or the wave just after it.

The fact that the tone of the duodenum sometimes rises several seconds before the gastric wave reaches the pylorus suggests strongly that some sort of a ripple travels out ahead of the visible wave. Such ripples, together with a shorter latent period at the sphincter (5) might account for the fact that the pylorus seems often to contract in the face of an advancing gastric wave, either blocking or reversing that wave.

Some of the differences in the sequence of the gastric and duodenal contractions suggest variations in the conduction time. There are also marked variations from time to time in the amount of conduction. Thus figure 6 *B* shows that the stomach can contract powerfully without producing any tone changes in the duodenum although such changes were well marked shortly before and shortly afterwards. Sometimes only one or two waves out of a series will fail to register in the duodenum. Similar observations have been made with the electrogastrographic technic.

The problem is complicated still further by the fact that the duodenum often shows rhythmic tonus changes when there are no corresponding contractions in the antrum detectable even with the galvanometer. (See fig. 1 *B*.) Our first thought was that impulses might be coming down from the fundus by way of the nerves, or that they might be carried over the antrum as ripples too shallow to show on our records, but further study made it seem more probable that the duodenum of itself tends from time to time to contract with a tonus

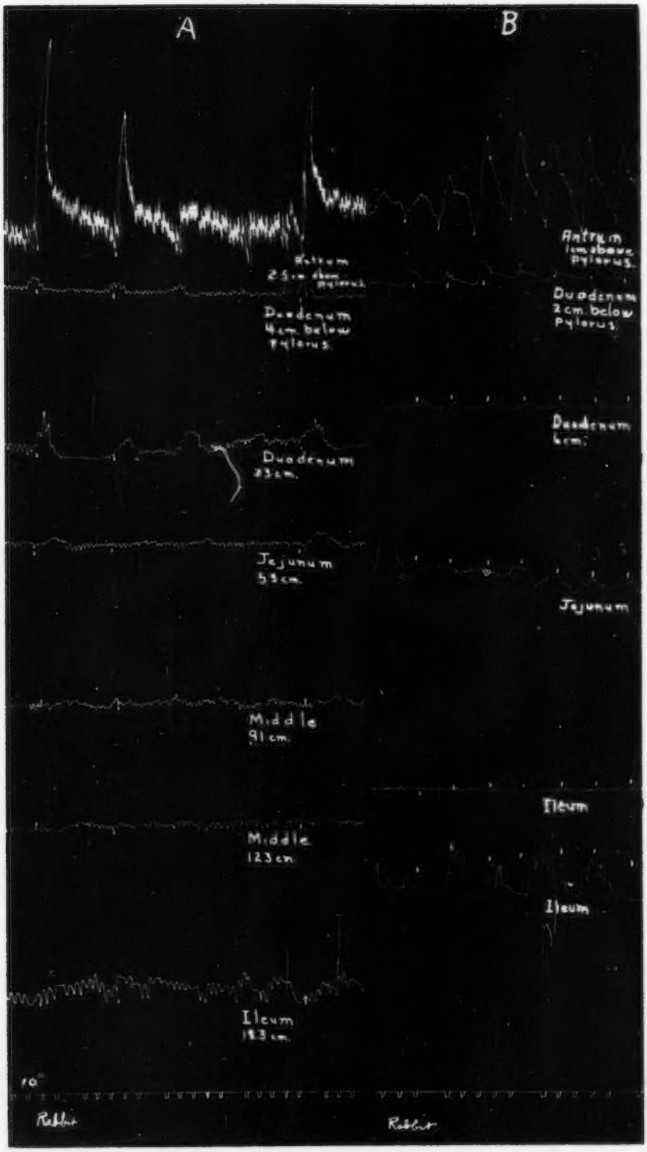

Fig. 5. Simultaneous mechanical records from the antrum pylori and from six parts of the small bowel in a rabbit. The movements of the longitudinal muscle are recorded. The marks show corresponding points. The time record shows 10-second intervals with breaks every minute. Note "tonus" waves as far down as the jejunum, apparently related to the waves in the stomach. Similar waves in the ileum are probably of local origin. Note in the upper left hand corner a duodenal wave appearing before the corresponding gastric one.

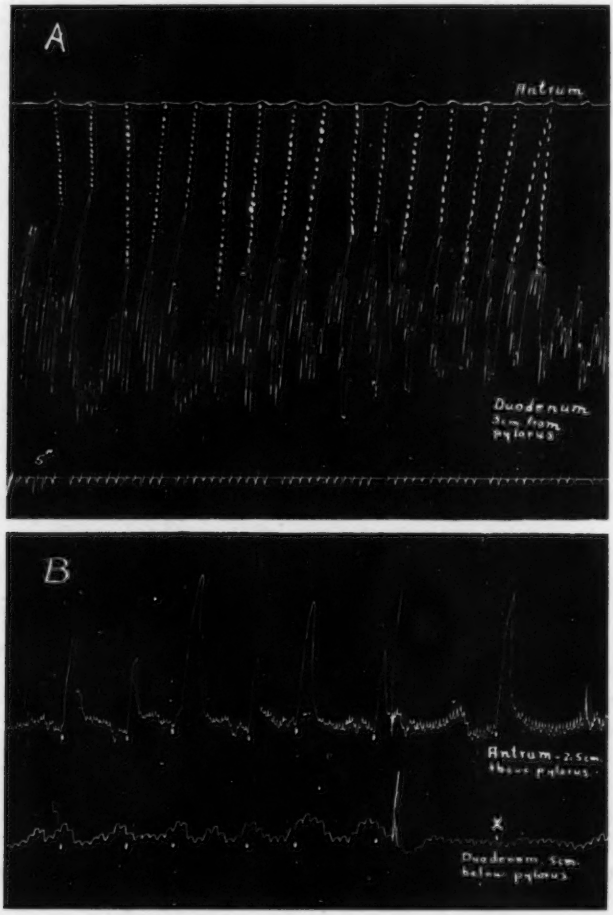

Fig. 6, *A*. Tracings from the antrum pylori and the duodenum of a rabbit. The dotted lines show that there is no relation between the gastric waves and the tonus changes in the duodenum.

Fig. 6, *B*. Similar tracings showing again that the tonus rhythm in the duodenum is largely if not entirely independent of that in the stomach. Note the failure of conduction at *X*.

rhythm about three per minute, a rhythm which can apparently be captured and dominated by the stomach. Sometimes, as in figure 6 *A*, it appears to be dominated, but a careful checking shows more or less independence; that is, in this instance there are at least sixteen duodenal waves to fifteen gastric ones; and if it had not been for temporary inhibitions, there probably would have been at least two more waves in the duodenum. The absolute independence of such rhythms is shown best by demonstrating them in excised segments of bowel, contracting rhythmically in warm Locke's solution. Figure 5 shows, furthermore, that they can be found in almost any part of the intact bowel.

It may be of interest to note at this point that we have had no difficulty in showing the sequence of waves in the stomach and duodenum in digestive tracts removed from the animals and placed in warm Locke's solution. That shows that the extrinsic nerves of the tract are not necessarily concerned in bringing about such conduction as occurs over the pyloric block.

There is some evidence that the duodenal contractions may affect and modify the activity of the stomach just as Wheelon and Thomas suspected. Thus we have noticed that when the duodenum is contracting powerfully in an effort to force its contents down the bowel the stomach seems often to come to its rescue at the right time with an unusually powerful wave or series of waves. After a rush has departed down the bowel, the duodenum often relaxes and quiets down as if fatigued from its efforts, and at the same time there occasionally is a temporary weakening or cessation of the gastric waves. Many of the mechanical records from the antrum show small waves corresponding to the duodenal ones. Although it is almost certain that these are produced by a slight tugging on the stomach by the bowel, it is hard to explain why they are sometimes more marked in the gastric than in the duodenal record. This point will have to be studied further. The small waves in the electrical antral record are most probably due to a spread of current from the duodenum.

The action currents of the pyloric region. As we have said before, we became particularly interested in this problem of the pyloric block when some two years ago we were able to get good galvanometric records from the digestive tract. As the technic has been touched upon in other articles (6) we will not go into details here. Suffice it to say that we have used small calomel electrodes, the mouths of which are held in close contact with the surface of the stomach or bowel by means of

little wooden or hard rubber clamps. These clamps are suspended so freely from a hook that the electrodes can easily follow the movements of the bowel, and there is no tendency for them to slip over the surface. Two d'Arsonval galvanometers record their deflections simultaneously on bromide paper carried on a moving drum. An upward movement of the light spot indicates negativity of the orad electrode. In order to get simultaneous mechanical records, one electrode is held firmly in a clamp while the other, a smaller lighter one, is allowed to move back and forth with the contracting muscles. Its movements are communicated to a delicate tambour and through rubber tubing to a little mirror on a Frank capsule.

Figure 2 *B* and *C* shows that the electrogram of the duodenum can exhibit "tonus" changes similar to those in the mechanogram, and that these have the same relations to the gastric activities as the mechanical ones have. At other times, the "tonus" waves are absent. As we have said before, some of the electrical records show such long time intervals between the gastric waves and the "tonus" waves that we can rule out a simple spread of current such as one can easily demonstrate by fastening the electrodes to a lobe of the liver. Another objection to the spread of current theory is that the rises were sometimes more marked in the duodenum 8 cm. below the pylorus than they were 2 cm. below it. Furthermore, figure 2 *A* shows that there may be very large voltage changes in the antrum without any sign of spread, either mechanically or electrically, to the duodenum.

Discussion. There can be no doubt now but that the muscle of the upper duodenum tends to shorten with the approach of each gastric wave to the pylorus. The question next arises: What does this shortening represent? Do the gastric waves actually jump the pyloric barrier, is there a spread of "tone"; or is there simply a relation between cycles such as Wheelon and Thomas describe? We do not care for the "cycle" terminology because we feel it needlessly complicates the description of what seems generally to be a simple wave-like progression. Similarly, to call the phenomenon "a spread of tone" does not elucidate matters. It would seem best, then, to say that although part, and sometimes all of the gastric wave is stopped at the pylorus, some of it tends to spread onward to produce a tone wave in the duodenum; a wave upon which are superimposed the small rhythmic contractions of the bowel. Occasionally this wave is associated with the extrusion of food from the stomach, and then it may give rise to a peristaltic rush down the intestine. Most of the time, however, there is no

expulsion of food from the stomach, and little or nothing to suggest that the tonus contraction of the upper duodenum is propagated down the bowel as a wave. In man, the wave coming from the stomach seems generally to jump over the first inch or so of the duodenum—the so-called cap. Better recording methods might, however, show a slight contraction of the thin muscle layer in that region also.

Another question which has been rather ignored by physiologists in the past is: Why should there be any block at all at the pylorus? In the first place, it might be ascribed to a considerable degree of discontinuity between the muscular coats of the stomach and duodenum. Aufschnaiter (7) has pointed out that the pyloric swelling is cut up into a number of sections by connective tissue partitions which run in planes at right angles to the long axis of the stomach. One of these partitions is quite dense and so complete that, in man, there is little muscular connection between the circular coats of the stomach and bowel. Toldt (8) believes that these partitions are remnants of the outer coat of the embryonic stomach, remnants which are left behind when the wall folds inward to form the pyloric selling. Most of the fibers in the outer longitudinal layer of the stomach dip down into the pyloric swelling to be inserted into these connective tissue partitions, so that, according to these anatomists, ordinarily only a few strands are left to run over on to the duodenum.

Unfortunately, very little work has been done upon this subject so that we know nothing about the generic and individual variations in this barrier in the different animals and man. As Cannon (9) has shown that the gastric waves can easily jump over connective tissue bands which are produced by making several circular cuts transversely around the stomach, it seems to us that we cannot explain the block purely on the basis of these anatomical barriers.

Another possible explanation might be looked for in the striking physiological difference between the muscle in the pyloric antrum of the stomach and in the duodenum. When segments are excised from these two regions, those from above tend to contract rhythmically three times a minute, while those from below contract about seventeen times per minute. It has long been known that a stimulus suited to the metabolism of a sluggish muscle will often have no effect on one which acts quickly, and which has a rapid metabolism; their "chronaxies" are different (10), and it may easily be that waves coming every 20 seconds cannot spread well into a tissue which contracts rhythmically every 3 seconds. It may be that the blocks which we have observed quite

frequently at the junction between the body of the stomach and the pars pylorica (6) are due to a similar transition from one type of muscle to another; from a pale soft tissue to a reddish, tough one. Similarly, in the excised tracts of cats and rabbits the lower end of the esophagus will contract rhythmically and powerfully for minutes at a time without sending off any waves over the adjacent stomach. The occurrence of such blocks in regions where there are no connective tissue partitions shows again that the partial barrier at the pylorus is not the essential factor in stopping the waves at that point.

In our opinion the pyloric block can best be explained in the following way. In the crop of Aplysia, Bottazzi (11) and von Brücke (12) have shown that the food does not press up against the lower sphincter. The real barrier is not the valve but a ring of muscle with high tone and rhythmicity placed around or just above it. This ring picks up stimuli ahead of its turn and thereby blocks or reverses approaching waves. Similarly, if one ties off the rectum of a cat and fills the colon with soapy water, powerful contractions soon appear which, from time to time, travel upward. As these waves approach the ileocecal sphincter it looks as if they would surely force it open, but suddenly the muscle there contracts and the oncoming wave either loses its stength and fades away, or else breaks ineffectually against the barrier. We have seen that an advance wave of some kind seems to run out well ahead of the visible one on the stomach, and there is some evidence (5) that the pyloric muscle is more sensitive than that in the rest of the antrum and that it has a shorter latent period. These observations could account, then, for the fact that in animals and in man the gastric waves often seem to fade away or get reversed just before they reach the sphincter. They might account also for the fact that many human stomachs fail to empty properly in spite of good peristalsis and a patent pylorus.

Any new information about the mechanics of the duodenal region is likely to have a large practical bearing because this part of the digestive tract has so much to do with the production of symptoms in disease. The investigation here reported may throw some light on the production of pain in duodenal ulcer because it seems most probable from all the evidence that such pains are due to muscular spasms and we see now that powerful gastric hunger contractions might easily produce disturbances in the duodenum. Unfortunately we cannot say yet why the gastric ulcers which presumably are exposed to the full force of the hunger contractions fail to give rise to the hunger pain so typical of duodenal ulcer.

Those who look for signs of Bayliss and Starling's law in our records will find little to help them. Occasionally there are signs of relaxation in front of a wave but such drops in the level of the record can just as well be due to the mechanical pull on the tissue by the muscle fibers contracting just above, or to the distention of the bowel by onrushing contents. Our findings agree with those of Wheelon and Thomas, whose published records show no sign of the workings of the law. The pyloric sphincter contracted the same no matter whether they stimulated the antrum above or the duodenum below. Unfortunatly, the law of the intestine is held to be so well proved that although these workers admit (13, p. 137) that their experiment "does not directly show" it, they later (p. 147) argue that they have in some way demonstrated its truth.

SUMMARY

Methods have been devised for obtaining simultaneous mechanograms and electrograms from several parts of the digestive tract.

Marked rises in the tone of the first portion of the duodenum often appear about the time the gastric waves reach the pylorus. Sometimes these "tonus" waves appear several seconds before the gastric wave approaches the sphincter.

Differences in conduction have been noted.

Rhythmic tonus changes often appear in the bowel when the stomach is quiet. They appear also in intestinal segments excised from the body and put into Locke's solution. This tonus rhythm is apparently captured at times by the stomach.

These phenomena can be demonstrated in the electrograms as well as in the mechanograms.

The peristaltic rushes in the bowel generally start from the duodenal tonus waves.

In man the duodenal contractions generally appear at the distal end of the "cap" a few seconds before a gastric wave reaches the pylorus.

Various explanations are offered for the presence of a considerable blockage at the pyloric line. It may be due to anatomic barriers; to differences in the muscle in stomach and bowel; or to a greater irritability of the pyloric ring which causes it to contract out of its turn.

Little or no evidence was found in favor of "the law of the intestine."

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THE INFLUENCE OF TEMPORARY CLOSURE OF THE RENAL ARTERY ON THE AMOUNT AND COMPOSITION OF URINE[1]

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An examination of textbooks, handbooks and monographs on physiology indicates that it is the general belief that temporary asphyxia of the kidney causes a cessation of urinary flow for a long time. This has also been frequently advanced as an argument against glomerular filtration. Brodie (1) in his Croonian lecture says "Yet another point which militates greatly against the idea that the glomerulus is a filter is the behavior of the kidney after temporary asphyxiation. If the renal artery be clamped for one minute and then released, the kidney does not at once begin to secrete, although the blood flow returns at once. It is only after a variable, but usually considerable delay that the kidney restarts, and at first the urine flow is very slow, only gradually returning to a rate comparable to the initial flow." In his excellent monograph on the secretion of urine, Cushny (2) states that "it has been demonstrated beyond discussion that anemia lasting for only a minute is sufficient to arrest the renal secretion for nearly an hour." Numerous other examples of this widespread belief could be given. In an attempt to produce anuria by short temporary asphyxia of the kidney, we experienced a complete failure and were unable to arrest the urinary flow for more than a minute or two even if the renal artery had been previously occluded for twenty minutes. This led us to examine the evidence for the above belief and then to investigate the subject experimentally.

Most of the statements concerning the effect of short temporary anemia on the kidney are based on Heidenhain's article in Hermann's

[1] A preliminary report of these results was Presented at the Chicago meeting of the American Physiological Society, December, 1920. See Proceedings, This Journal, 1921, lv, 278.

Handbuch (3). He gives here as his authority a reference to the early work of Hermann in Ludwig's laboratory. However, if one reads carefully the article referred to, one finds that Hermann does not state that clamping the renal artery for one minute produced a long cessation of urine flow. Hermann (4) writes "Wenn Man die Arterie einen Moment zusammendrückt, so ändert sich der Harn nicht. Derselbe enthält aber sogleich Eiweiss, wenn der Verschluss nur einige Secunden gedauert hat." And again "Wenn der Verschluss der Arterie längere Zeit gedauert hat, so bleibt der Harn anfangs gerwöhnlich aus und wird später sehr langsam ausgeschieden." He states that the occlusion lasted for "1–24 stunden." This obviously is a misprint as his protocols give urine quantities 5 minutes after commencing to clamp the artery. Overbeck (5), a year later, gives a protocol where the left renal artery was clamped for one minute and urine flow is recorded as occurring five minutes later. Asher (6) states that Ludwig found that clamping the renal artery for one minute caused a cessation of the flow of urine for hours, but gives no reference. Working with rabbits, he finds that clamping of the renal artery for one minute stops the urine flow and that it is not reinstated by the administration of diuretics during the period of observation (about 45 minutes). Carter (7), in a paper on the elimination of dyes by the kidney, states that cessation of the blood flow for one-half to one minute caused a temporary cessation of urine flow, but that it frequently restarts on the injection of fluid. Barcroft and Straub (8) note that the kidney survives the operation of clamping the vessels for 15 minutes when the blood has been washed out with Ringer's solution. Barcroft (9) states that the kidney can be made to secrete after temporary cessation of the blood flow for periods of 10 seconds to 18 minutes by the administration of sodium chloride or Ringer's solution. This, it is to be noted, is directly opposed to the observations of Asher quoted above.

The above brief review of the experimental observations would appear by no means to justify the definite statements which are universally made in regard to the effect of short temporary anemia of the kidney. The experiments which we have undertaken prove definitely that short temporary anemia of the kidney does not cause a cessation or diminution of urine flow; and, moreover, offer a possible explanation for the somewhat conflicting results obtained by others. After establishing the fact that asphyxia of several minutes' duration did not stop or necessarily reduce the elimination of water, it appeared possible to use this method in an attempt to differentiate between those bodies

which are eliminated by the kidney entirely by mechanical filtration and those which might be eliminated by the activity of the epithelial cells. How far we have been successful will be recorded in the succeeding pages.

Methods. Dogs were used in all experiments except in a few which were made on rabbits to see if a species variation might account for some of the conflicting results in the literature. The dogs were anesthetized with paraldehyde, the rabbits, with urethane. The ureters were exposed through the flank without opening the peritoneal cavity. The left renal artery was always selected for clamping, and was carefully exposed extraperitoneally by an incision an inch or two above the incision for the ureter. It was found difficult to prepare the artery for clamping without injuring the nerves of the renal plexus. Although this was attempted in certain experiments, in others, in which the left splanchnic nerve had been sectioned, no attempt to preserve the renal nerves was made. It was noticed early in the course of the work that handling or manipulating the renal pedicle might produce a diminution of urine flow or even a short anuria. As this seriously interfered with the interpretation of the result of clamping the renal artery, it was more or less avoided in many experiments by sectioning the left splanchnic nerve. This suggests that it is due to a reflex vasoconstriction of the renal vessels. In two or three experiments in which the ureters were cannulated and the renal artery exposed through an abdominal incision, occlusion of the artery by means of a ligature placed around the vessel and then tightened resulted in a marked diminution or stoppage of urine flow after the pressure was released. Complete removal of the ligature, however, resulted in a marked improvement of the flow; and, in view of our other results obtained by the other method of exposure of the ureters and artery, we concluded that something else besides the temporary anemia was responsible for the oliguria or anuria. These sources of error have undoubtedly misled others who have studied the problem. The renal artery was occluded by means of either a small clamp or for short periods by means of the thumb and finger.

The urine specimens from each kidney were collected in tall narrow graduated cylinders, measured, and then diluted to an appropriate volume. The various urinary constituents were estimated by the methods already referred to in a previous article by Marshall and Crane (10).

Results. The following are protocols of typical experiments selected from many in which the renal artery was occluded for short periods (from 1 to 5 minutes). The urine flow usually commences immediately after the circulation is reëstablished; occasionally, as long a period as a minute may elapse before signs of urinary flow can be detected. The time at which the commencement of urinary flow after the clamping is given in the protocols is the time when the first drop of urine fell from the ureteral cannula. Obviously, this will depend on the rate of excretion. Thus, with a slow rate of flow, the time for the formation of a drop will be much longer than with a more rapid rate of secretion.

Very little, if any, demonstrable effect on the elimination of water and the various urinary constituents studied is evident. The urine obtained from the kidney which has been subjected to short temporary anemia contains protein, but this also disappears completely from the urine within a half-hour. It is obvious that we here have the appearance of protein in the urine with no other observable functional change in the kidney; the kidney is equally efficient in regard to its function as the normal kidney.

Experiment 1. Dog, male, weight 7.3 kilos. February 13, 1920. Ureters cannulated, and left renal artery exposed. Left renal artery clamped from 12:11:30 to 12:14:30. Drop fell at 12:17:00. Artery again clamped from 1:19 to 1:26, and left commenced flowing at 1:28.

TIME		URINE	CREATININE	TIME		URINE	CREATININE
		cc.	*mgm.*			*cc.*	*mgm.*
11:40–12:10	R	1.5	1.98	12:48–1:18	R	1.2	1.86
	L	1.7	2.19		L	1.8	1.86
	L/R	1.13	1.10		L/R	1.50	1.00
12:18–12:48	R	1.4	1.89	1:29–1:59	R	1.1	1.96
	L	2.0	2.19		L	1.9	2.18
	L/R	1.43	1.16		L/R	1.73	1.11

Experiment 2. April 28, 1920. Dog, female, weight 8.4 kilos. Ureters cannulated. Left renal artery exposed. At 12:33, injected 20 cc. of a solution containing 6 per cent sodium chloride, 3 per cent urea and 0.3 per cent creatinine. At 1:14 and at 2:00, 5 cc. more of diuretic mixture given. Left renal artery clamped from 1:18 to 1:19, urine flowed 5 seconds after removing clamp. Again clamped from 2:01 to 2:04, with urine flow commencing 30 seconds after release.

TIME		URINE	NaCl	UREA	CREATININE
		cc.	*mgm.*	*mgm.*	*mgm.*
12:55–1:15	R	5.8	62.5	108	8.81
	L	6.7	70.0	107	8.58
1:20–1:40	R	6.5	70.0	83	7.00
	L	7.5	82.5	86	7.11
1:40–2:00	R	6.0	70.0	83	4.69
	L	9.0	92.5	95	4.80
2:05–2:25	R	9.6	113.0	89	6.71
	L	13.0	125.0	93	7.25

Experiment 3. September 28, 1920. Dog, male, weight 6.0 kilos. Ureters cannulated, left renal artery exposed. At 10:59, 11:06 and 12:15, given 10 cc. of 10 per cent sodium chloride solution. Artery clamped from 12:17:30 to 12:20:30, flow of urine started 10 seconds after release.

TIME		URINE	NaCl	UREA	NH_4	CREATININE	PROTEIN
		cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
11:17–11:47	R	6.2	65.0	19.0	1.53	1.15	–
	L	6.0	72.0	20.0	1.59	1.17	–
	$\frac{L}{R}$	0.97	1.10	1.05	1.04	1.02	
11:47–12:17	R	5.2	62.0	18.0	2.24	1.18	–
	L	5.5	59.0	17.0	2.20	1.20	–
	$\frac{L}{R}$	1.06	0.95	0.95	0.98	1.02	
12:25–12:55	R	7.7	92.0	21.0	2.04	1.14	–
	L	7.5	84.0	18.0	1.84	1.07	+
	$\frac{L}{R}$	0.98	0.91	0.86	0.90	0.94	
12:55– 1:25	R	7.7	105.0	21.0	1.73	1.14	–
	L	7.8	105.0	19.0	1.63	1.05	–
	$\frac{L}{R}$	1.01	1.00	0.90	0.94	0.92	

The following protocol is illustrative of the fact that simply opening the abdomen in rabbits and handling the kidney and intestines may

produce a unilateral anuria. This probably explains the results of Asher's experiments on clamping the renal artery.

Experiment 4. May 24, 1920. Rabbit 1.42 kilos. Tracheal tube inserted and jugular vein cannulated. Left renal artery exposed by incision through left rectus. Ureters cannulated through midline abdominal incision. Operations finished at 11:54. At 11:55, injected intravenously 15 cc. of 5 per cent sodium chloride. Urine flowing from both ureters, but left is flowing much more slowly than right. Left gradually approaches right in rate. At 1:40, flow about equal from left and right. Given 20 mgm. of theocin.

1:52–2:01. Right and left equal in amount, 3.7 and 3.6 cc. respectively.

2:03–2:04. Opened abdominal incision, exposing left renal artery, artery neither touched nor occluded, but kidney and intestines handled.

2:05–2:56. Right has flowed 5.3 cc., left only a few drops.

It is obvious that the effects of exposing the intestine and kidney on the urine flow must be avoided in order to study the effect of clamping the artery. If a rabbit is eviscerated, the renal artery of either side can with care be occluded without any manipulation or handling of the artery or kidney. The two experiments given below show that simply clamping of the artery for 1 minute or less has no influence on the rate of urine flow, and that even 20 minutes' anemia does not produce anuria.

Experiment 5. May 28, 1920. Rabbit, female, 1.70 kilos. Anesthetized with urethane. Tracheal tube and cannula in external jugular vein inserted. Stomach, intestines, spleen and pancreas removed. Ureters cannulated, both flowed at once. Rate of urine flow measured by counting the drops from each cannula for 3-minute periods. Small quantities of 5 per cent sodium chloride injected occasionally to produce diuresis.

12:33–12:36. Right, 17 drops; left, 13 drops.

Left renal artery clamped for 10 seconds.

12:36–12:39. Right, 13; left, 7.

12:39–12:42. Right, 8; left, 8.

12:43–12:46. Right, 9; left, 8.

12:46–12:49. Right, 5; left, 4.

12:49–12:52. Right, 4; left, 5.

Left renal artery clamped for 30 seconds.

12:52–12:55. Right, 4; left, 12.

12:55–12:58. Right, 3; left, 11.

Experiment 6. April 27, 1921. Rabbit, 2.3 kilos. Stomach, intestines, spleen and pancreas removed. Left external jugular vein cannulated. Ureters cannulated. Five per cent sodium chloride solution injected to produce diuresis.

3:42–3:57. Right, 8.3 cc.; left, 4.8 cc.

4:00–4:20. Right renal artery clamped.

First drop of urine fell at 4:20:30, second drop at 4:25:30, third at 4:26:30.

4:27–4:42. Right 3.0 cc.; left, 3.8 cc.

It having been established that clamping the renal artery for short periods (1 to 5 minutes) does not disturb the function of the kidney, it was of interest to inquire what effect would be produced by longer periods of anemia. It is quite clear from the results which we have obtained that a cessation of the blood flow through the kidney for twenty minutes does not inhibit the flow of urine when the circulation is reëstablished, and generally does not reduce the elimination of water. On account of the unavoidable injury to the nerve fibers of the renal plexus during the exposure and clamping of the renal artery, the splanchnic nerve on the left side was frequently sectioned before the experiment in an attempt to make the conditions before and after clamping the artery more comparable. This, however, requires an understanding of the effects of section of this nerve in order properly to interpret the results. The effects of unilateral section of the splanchnic nerve have been carefully studied by Marshall and Kolls (11) and Marshall and Crane (10). The increased blood flow through the kidney after section of the splanchnic nerve produces a marked increase in the elimination of water, chlorides and carbonates, a much slighter increase in that of urea, phosphates and sulphates, and no change in that of creatinine and ammonia. In addition, the ratio between the amounts of water eliminated by the two kidneys under these conditions is by no means constant. It is necessary, therefore, to take into consideration the relative ratios of the volumes of urine from the two kidneys before and after occlusion of the artery in interpreting an increase or decrease in the various urinary constituents. Since creatinine and ammonia are not influenced by increased blood flow no account of water changes need be considered in considering decreases of these substances; however, since chlorides and carbonates are greatly influenced, changes in the urine volume ratio of the kidneys will markedly influence the ratios of these substances. Due to the further fact that no simple relation always exists between these factors, it is very difficult to determine the magnitude of the effect on the different urinary constituents. Be that as it may, it is quite evident from an examination of the representative protocols and summaries of the experimental findings given below, that one can safely draw certain conclusions.

Experiment 7. September 27, 1920. Dog, male, weight 10.9 kilos. Ureters cannulated, left renal artery exposed. At 11:15 and 11:53, 10 cc. of 10 per cent sodium chloride solution given intravenously. Artery clamped from 12:26 to 12:36, urine flow commenced within 1 minute after release.

TIME		URINE	NaCl	CREATININE
		cc.	*mgm.*	*mgm.*
11:23–11:53	R	6.7	112.0	3.26
	L	5.6	102.0	3.20
	$\frac{L}{R}$	0.84	0.91	0.98
11:54–12:24	R	4.7	90.0	3.56
	L	4.7	88.0	3.84
	$\frac{L}{R}$	1.00	0.98	1.08
12:41– 1:11	R	4.0	64.0	4.16
	L	7.4	70.0	3.16
	$\frac{L}{R}$	1.85	1.09	0.76
2:11– 2:41	R	3.6	46.0	4.00
	L	3.5	36.0	3.92
	$\frac{L}{R}$	0.97	0.78	0.98

Experiment 8. September 30, 1920. Dog, female, weight 12.2 kilos. Left renal artery exposed and ureters cannulated. At 11:15, given 20 cc. of 10 per cent sodium chloride solution intravenously, and at 12:05, 10 cc. more. Left renal artery clamped from 12:26:30 to 12:46:30. Commenced to flow within 2 minutes after release.

TIME		URINE	NaCl	UREA	NH_4	CREATININE
		cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*
11:25–11:55	R	6.2	73.0	61.0	3.06	2.63
	L	9.0	93.0	76.0	2.72	2.63
	$\frac{L}{R}$	1.45	1.28	1.25	0.90	1.00
11:55–12:25	R	3.3	33.0	92.0	4.93	3.08
	L	5.9	48.0	108.0	4.84	3.28
	$\frac{L}{R}$	1.80	1.45	1.18	0.98	1.06
12:50– 1:20	R	4.2	30.0	110.0	6.29	3.82
	L	8.0	68.0	65.0	1.53	2.51
	$\frac{L}{R}$	1.90	2.27	0.59	0.24	0.66
1:20– 1:50	R	4.0	34.0	117.0	6.00	3.98
	L	9.5	103.0	88.0	1.70	3.32
	$\frac{L}{R}$	2.38	3.03	0.75	0.28	0.83

Experiment 9. November 10, 1920. Dog, male, weight, 11.0 kilos. Left splanchnic nerve sectioned. Ureters cannulated, and left renal artery exposed. At 1:22, injected 20 cc. of 10 per cent sodium chloride solution; and at 3:30, 10 cc. of the same solution. Artery clamped from 3:35 to 3:55. Urine commenced to flow within 2 minutes after removing clamp.

TIME		URINE	NaCl	UREA	CREATININE	NH_4	PO_4	SO_4
		cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
1:30–2:32	R	13.1	178.0	165.0	7.44	4.36	36.0	12.1
	L	24.0	328.0	239.0	8.00	4.36	45.5	15.5
	$\frac{L}{R}$	1.83	1.88	1.45	1.07	1.00	1.26	1.28
2:32–3:32	R	5.1	64.0	194.0	7.20	4.84	22.0	7.0
	L	23.2	285.0	262.0	7.04	5.45	34.0	10.5
	$\frac{L}{R}$	4.55	4.45	1.35	0.98	1.13	1.55	1.50
3:58–5:18	R	9.5	108.0	256.0	8.40	6.54	63.0	16.1
	L	39.5	334.0	238.0	6.88	3.52	68.0	16.8
	$\frac{L}{R}$	4.16	3.09	0.93	0.82	0.54	1.08	1.04
5:18–6:18	R	5.9	41.0	193.0	6.40	6.17	65.0	10.2
	L	17.0	120.0	170.0	5.28	4.22	45.0	6.2
	$\frac{L}{R}$	2.88	2.93	0.88	0.83	0.68	0.72	0.61

Experiment 10. December 21, 1920. Dog, male, weight 17.3 kilos. Left splanchnic nerve sectioned. Ureters cannulated and left renal artery exposed. At 12:50, given 20 cc. of 10 per cent sodium chloride solution. From 1:41 to 2:06, artery clamped, urine commenced to flow 3 minutes after release.

TIME		URINE	NaCl	PO_4	SO_4	CREATININE
		cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*
12:58–1:38	R	6.0	65.0	44.0	16.7	9.1
	L	50.0	650.0	73.0	29.5	11.3
	$\frac{L}{R}$	8.34	10.0	1.66	1.77	1.24
2:10–2:50	R	14.5	150.0	54.0	29.1	14.5
	L	50.6	500.0	42.0	7.8	7.7
	$\frac{L}{R}$	3.49	3.33	0.78	0.30	0.53
2:50–3:33	R	14.0	130.0	35.0	26.6	12.5
	L	43.0	514.0	31.0	12.4	9.5
	$\frac{L}{R}$	3.07	3.33	0.89	0.47	0.76

An examination of the above protocols indicates that anemia of 20 or 25 minutes' duration markedly influences the elimination of various substances by the kidney. It is also clear that the damage is not permanent and partial recovery can be noted in the subsequent periods of many of the experiments. The disturbance to the elimination of different substances is by no means the same. Thus, it is to be noted that the elimination of water and chlorides may be unchanged or even slightly increased, while the elimination of urea, phosphates, sulphates, creatinine, and ammonia is markedly diminished. The elimination of ammonia appears to be much more markedly depressed by the previous anemia than that of the other bodies. It is interesting to note that the total elimination of creatinine and ammonia appears to be as great as in the normal periods, the decrease in elimination by the damaged kidney being balanced by an increase in that of the normal organ.

The following protocols are representative of experiments carried out on the elimination of carbonates. The special precautions in the collection of urine shown to be necessary in this type of experiment (12) were all observed. The total carbon dioxide of the urine was determined, but a determination of the hydrogen ion concentration of the urine permits us to calculate the ratio of free to bound carbon dioxide (bicarbonate). If we assume the bicarbonate to be completely ionized, and use 4.4×10^{-7} for the dissociation constant of carbonic acid (13), the results are sufficiently accurate for the present purpose. The results on the elimination of bicarbonate indicate clearly that the amount of bicarbonate eliminated is markedly increased after temporary anemia of the kidney. This is a distinct contrast to the decrease in phosphate and creatinine observed in the same experiments. The hydrogen ion concentration is decreased in the urine obtained after clamping the renal artery. Calculation from the data given in the protocols shows that the percentage of free carbon dioxide in the urine from the injured kidney is much greater than in that from the normal side. This would suggest strongly that the tension of carbon dioxide is much greater in the kidney subjected to anemia than in the normal organ. In seeking an explanation for the increased elimination of carbonates after the injury, the data of experiment 13 are very suggestive. Here it is noticed that if bicarbonate is injected into the blood in large amounts, temporary anemia does not increase the amount of bicarbonate elimination. This will be discussed more fully in a subsequent place.

Experiment 11. May 18, 1921. Dog, male, weight 17.0 kilos. Left splanchnic nerve sectioned. Ureters cannulated with long bent cannulae so as to collect urine under oil. Left renal artery exposed. At 11:10, given 40 cc. of 10 per cent sodium chloride. From 12:56 to 1:16, artery clamped, and urine flow commenced within 1 minute after release.

TIME		URINE	TOTAL CO_2	BOUND CO_2	pH	PO_4	CREATININE
		cc.	*mgm.*	*mgm.*		*mgm.*	*mgm.*
12:23–12:53	R	6.0	0.62	0.15	5.9	28.0	7.50
	L	15.0	1.46	0.52	6.1	35.0	6.90
	$\frac{L}{R}$	2.50	2.35	3.46		1.25	0.92
1:18– 1:48	R	9.2	1.15	0.25	5.8	51.0	8.25
	L	15.0	11.22	5.26	6.3	38.0	5.40
	$\frac{L}{R}$	1.63	9.76	21.1		0.75	0.65
1:48– 2:18	R	6.2	0.89	0.27	6.0	41.0	6.80
	L	18.6	12.75	9.85	6.9	31.0	5.35
	$\frac{L}{R}$	3.00	14.3	36.5		0.76	0.79

Experiment 12. May 27, 1921. Dog, male, weight 15.5 kilos. Left splanchnic sectioned, and left renal artery exposed. Ureters cannulated with long bent cannulae so that urine could be collected under oil. Given 20 cc. of 10 per cent sodium chloride. From 1:10 to 1:30 artery clamped, urine commenced to flow 10 seconds after release.

TIME		URINE	TOTAL CO_2	BOUND CO_2	pH	PO_4	CREATININE
		cc.	*mgm.*	*mgm.*		*mgm.*	*mgm.*
12:37–1:07	R	16.5	1.93	0.59	6.0	30.0	13.5
	L	36.0	3.77	1.55	6.2	37.5	16.0
	$\frac{L}{R}$	2.18	1.95	2.63		1.25	1.18
1:33–2:03	R	18.5	1.74	0.26	5.6	31.6	13.5
	L	37.0	42.4	32.7	6.9	26.7	8.4
	$\frac{L}{R}$	2.00	24.4	125.5		0.84	0.62

Experiment 13. July 11, 1922. Dog, male, weight 10.7 kilos. Left splanchnic sectioned, and left renal artery exposed. Ureters cannulated with long bent cannulae to collect urine under oil. Given 60 cc. of 10 per cent sodium bicarbonate intravenously. From 10:41 to 11:01, artery clamped. Urine flowed within 2 minutes after release.

TIME		URINE	TOTAL CO_2
		cc.	*mgm.*
10:20–10:40	R	10.0	54.4
	L	18.4	70.5
	$\frac{L}{R}$	1.84	1.30
11:04–11:34	R	19.0	114.0
	L	26.5	115.0
	$\frac{L}{R}$	1.40	1.01

The protocols and summaries which have been given are representative of most of the experiments which have been performed in the course of the present investigation. It has been mentioned that it was frequently observed that the handling of the renal artery necessary to the preliminary exposure for clamping produced a depression in the elimination of urine. Likewise, the manipulation needed to place a clamp upon the artery might produce a decrease in the amount of urine eliminated. After we had become convinced that this effect was not due to a temporary cessation of the blood flow, and since it seriously interfered with the interpretation of the result of temporary anemia, efforts were made to abolish or lessen this effect. It was observed that the depth of anesthesia apparently had some bearing upon producing a diminution of flow from this cause. Also, this depression was generally absent if the splanchnic nerve had been previously sectioned. Great care and gentleness in exposing the artery and applying the clamp also lessened its occurrence. An examination of all of our records in which the left renal artery was occluded for a period of 20 or 25 minutes gives the following data as to the occurrence of a lessened flow of urine after clamping. Of five experiments, in which the splanchnic nerve was not sectioned, four showed a decrease in the water elimination; while of 14 cases, where the nerve was sectioned, only 3 showed a depression of the water elimination after clamping. We believe that this is probably due to a reflex constriction of the renal vessels and is not an effect of the temporary anemia. The following protocol is typical of this type of result. The decrease in urea and creatinine after anemia is distinctly more marked than is that of water or salt, which is just the reverse of what occurs when the amount of urine is reduced by diminishing the blood flow through the kidney (14).

Experiment 14. April 28, 1920. Dog, female, weight 8.4 kilos. Ureters cannulated, and left renal artery exposed for clamping. A solution of urea 3 per cent, creatinine 0.3 per cent, and sodium chloride 6 per cent injected intravenously to produce diuresis. Left renal artery occluded from 2:47 to 3:07. Urine commenced to flow within 2 minutes after releasing artery.

TIME		URINE	NaCl	UREA	CREATININE
		cc.	*mgm.*	*mgm.*	*mgm.*
2:25–2:45	R	5.8	65.0	78.0	4.02
	L	9.5	98.0	89.0	4.48
	$\frac{L}{R}$	1.64	1.51	1.14	1.11
3:10–3:30	R	6.3	70.0	110.0	7.58
	L	3.5	43.0	30.0	2.42
	$\frac{L}{R}$	0.56	0.61	0.27	0.32
3:50–4:10	R	5.5	73.0	104.0	6.62
	L	4.5	38.0	62.0	4.29
	$\frac{L}{R}$	0.82	0.52	0.60	0.66

The following protocol is one which illustrates a discrepancy in the results which has been only observed twice. It shows an apparent increase in the elimination of phosphate after a temporary anemia of 20 minutes' duration. We have no explanation to offer at present.

Experiment 15. December 14, 1920. Dog, female, weight 12.0 kilos. Left splanchnic nerve sectioned. Left renal artery exposed and ureters cannulated. Ten per cent sodium chloride solution injected intravenously to produce diuresis. From 3:00 to 3:21, clamped left renal artery. Urine commenced to flow within 1 minute after release.

TIME		URINE	PO_4	CREATININE
		cc.	*mgm.*	*mgm.*
2:38–2:58	R	7.5	10.6	2.54
	L	24.8	19.0	3.18
	$\frac{L}{R}$	3.30	1.79	1.25
3:23–3:43	R	5.5	7.0	3.10
	L	25.5	20.6	2.54
	$\frac{L}{R}$	4.63	2.95	0.82

An examination of the two kidneys from a great number of the animals used in these experiments, after fixing in formaldehyde or Zenker's fluid, sectioning and staining by the usual methods, was very kindly made for us by Prof. E. L. Opie, of the Pathological Department of Washington University. However, it is needless to describe his reports in detail as the final conclusion was that no noteworthy difference between the kidneys on the two sides was noted.

Discussion. The experimental results which have been presented establish clearly the fact that cessation of the blood flow through the kidney for short periods of time does not produce a prolonged anuria, and produces no change in the excretion of the kidney except the appearance of protein in the urine. Consequently, arguments against filtration which have been based upon the belief that a few minutes' closure of the renal artery produces a prolonged cessation of urine flow do not hold due to the inaccuracy of the data upon which they are based. It is true that one might expect injury to the cells to be followed by greater permeability and increased filtration, as is found with the production of lymph. In certain instances the data do suggest an increased elimination of water after injury, but it is by no means a marked or constant finding. On the other hand, it would be necessary to know whether the vascular conditions in the kidney are the same after injury as before to determine whether an increased permeability had been produced. From the nature of the experiment, we know that the composition of the blood supplied to the two kidneys is the same, any change in this factor after clamping is taken into account by utilizing the normal kidney as a control. Barcroft (9) presents data indicating that the blood flow through the kidney is much diminished after an anemia of twenty minutes, but then the diuresis after injury was not as great as before. It is possible that the escape of the large molecules of plasma protein through the glomerular membrane, which normally retains them, may affect the passage of the smaller molecules. At any rate, the conditions are not analogous to the production of lymph which normally contains a large amount of colloids. It is evident, however, that the experiment of clamping the renal artery for a minute or so cannot be used as an argument against filtration, but that our results are entirely in accord with the filtration theory of glomerular activity.

Anemia, of twenty minutes' duration produces under the conditions of our experiments the following result. The elimination of water and chloride is little influenced, that of bicarbonate is markedly increased

while the absolute amounts of urea, phosphate, sulphate, creatinine and ammonia are all reduced. A division of the substances eliminated by the kidney can then be made on the basis of the effect of temporary asphyxia. One group, including water, chloride and bicarbonate is uninfluenced or increased; while a second group, including the other substances, is distinctly diminished as a result of this injury to the kidney. The first group includes substances which are present in the plasma in relatively large amounts and possess a high threshold; the other includes the substances which are present in relatively small amounts in the blood and have a very low threshold. Other characteristics of the first group of bodies distinguishing them from the second are that their excretion is more markedly influenced by changes in blood flow through the kidney (10) and more depressed by back pressure in the ureter than the substances of the second group (15).

It is, no doubt, possible to explain these results on the theory of filtration and reabsorption being the only processes concerned in the elimination of urine by the kidney, provided the reabsorption by the tubules is markedly changed in character by the anemia. However, in two recent communications (16) we have presented evidence that filtration and reabsorption alone are insufficient to explain the excretion of urine; and that secretion and synthesis of certain substances take place in the tubules. The simplest explanation of the results which are under discussion would appear to be that filtration is but little influenced by the anemia, but that the more specialized process of secretion is seriously interfered with. Water, chloride and bicarbonate are then eliminated entirely by filtration along with the other non-colloid constituents of the plasma, while urea, phosphate, sulphate, and creatinine[2] are mainly excreted by a process of secretion. The fact that ammonia is decreased much more in amount than the other bodies of the same group is possibly related to the finding of Nash and Benedict (17) that the ammonia of the urine is formed in the kidney. The marked increase in the bicarbonate is most easily interpreted as a decrease in the reabsorption of this substance in its passage along the tubules. The fact that the increase does not take place when the blood concentration of bicarbonate is increased and large amounts are being eliminated would appear to support this view. In this case we might argue that very little reabsorption of the bicarbonate is normally taking place; hence, difficulty of reabsorption could not increase its elimination. One might naturally expect the chloride to be in-

[2] The recent work of Behre and Benedict (18), if confirmed, would indicate that creatinine, like ammonia, is formed by the kidney.

creased in the same way as the bicarbonate on account of lack of reabsorption. In practically all our experiments reported here, the chloride of the plasma has been increased by the injection of sodium chloride to produce diuresis, so that the condition corresponds to the experiment (no. 13) where bicarbonate excretion is not increased. Certain data, not included here, would seem to indicate that the reabsorption of chloride is not interfered with as much as that of bicarbonate, and this would correspond to the findings of Carr (19) in a study of water diuresis. He observed that the bicarbonate of the urine was increased during water diuresis in percentage as well as absolute quantity, while the chloride percentage was markedly reduced although the absolute amount eliminated might be slightly increased.

The experiments reported in this paper, in conjunction with other data on urinary secretion would appear to be best explained by the following outline of renal excretion. All of the non-colloid constituents of the plasma are eliminated by filtration through the glomerulus; urea, phosphate, sulphate and other bodies are added to this filtrate by secretion of the tubular epithelium; water, chloride, bicarbonate and other substances are reabsorbed during their passage along the tubule; and, lastly, certain substances which are formed in the kidney, ammonia (and perhaps creatinine) and other bodies, are secreted by the tubules. Chloride and bicarbonate are eliminated entirely by filtration, and are reabsorbed under certain conditions almost entirely in passage along the tubule. While the extent of the reabsorption of chloride probably depends mainly on the concentration of chloride in the plasma, the amount or reabsorption of bicarbonate taking place is certainly conditioned largely by changes in the hydrogen ion concentration of the blood. Thus, it has been shown (20) that large amounts of bicarbonate are eliminated in the urine during forced breathing, although the concentration of this substance in the plasma is much below the normal value. The plasma under these conditions is, however, more alkaline. The fact that the concentration of bicarbonate in the urine varies with the hydrogen ion concentration (12) is also confirmatory of this view.

It is interesting to note that Davies, Haldane and Peskett (21) from a study of the maximum concentrations of chloride and bicarbonate in man, and the effect of urea and phosphate elimination on these maxima reach a similar conclusion to ours in regard to the elimination of these substances. Their findings led them to believe that chlorides and bicarbonates are concentrated by reabsorption, urea and phosphates by secretion.

SUMMARY

The widespread belief that an anemia of the kidney of short duration produces a prolonged anuria has been found to be untrue. Any arguments which have been used against glomerular filtration based on this belief, consequently, do not hold. Certain explanations are suggested from our experiments to explain the discrepancies observed by previous workers.

A cessation of the blood flow through the kidney for a short period (1 to 3 minutes) produces no effect on the excretion of the kidney when the circulation is reëstablished, except the appearance of protein in the urine. Anemia of longer duration (20 to 25 minutes) produces definite changes in the urine eliminated by the kidney. These changes may be summed up as follows: The elimination of water, chloride and bicarbonate is unchanged or may be increased, while that of urea, phosphate, sulphate, creatinine and ammonia is distinctly decreased.

These results, in conjunction with other data on renal secretion, appear to be best explained by the theory of urinary secretion which embraces glomerular filtration, reabsorption of fluid and certain substances by the tubules, and the secretion of various bodies by the tubular epithelium. As far as is possible at present, the details of such a theory have been discussed.

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